VOL. VI OCTOBER, 1918 No. 4

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PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter August 23, 1917, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

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Price { $6.00 per year, two volumes, United States
$6.25 per year, two volumes, Canada
$6.50 per year, two volumes, other countries

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THE PRESENCE OF ALUMINUM AS A REASON FOR THE DIFFERENCE IN THE EFFECT OF SO-CALLED ACID SOIL ON BARLEY AND RYE[1]

BURT L. HARTWELL AND F. R. PEMBER

Rhode Island Agricultural Experiment Station

Received for publication October 19, 1918

INTRODUCTION

It has been found by means of field experiments conducted at the Rhode Island station that under conditions where liming exerted very little influence upon the growth of rye, barley was increased two to threefold (4). These crops were selected in the present instance simply as convenient representatives of crops differently affected by acid-soil conditions.

In a previous article by us (5), the method of water culture there described being the same as in the present instance, it was shown that barley seedlings were not more susceptible than rye seedlings to injury by acidified nutrient solutions, even though so-called acid soils are much more deleterious to barley than to rye. The reason for the different effect of acid soil and acid nutrient solutions has interested the authors for a number of years. The present paper is a record of their intermittent work on the subject.

Unless otherwise stated, the solution work with seedlings was conducted in 250 cc. wide-mouthed bottles under such conditions that the transpiration could be measured during the few weeks of the experimental period. The total transpiration could be compared with the final weight of the green tops.

EFFECT OF ACID IN WATER AND SAND CULTURES

There are so many different cultures involved in the present work that a condensation of the data seems desirable. Therefore, in most cases the transpiration and weight of the green tops accompanying each bottle are not given, but the effect of a stated treatment is considered to be shown by comparing the average of both these observations derived from duplicate cultures. For example, in recording some additional data concerning the relative effect on rye and barley seedlings of sulfuric acid added to the nutrient solution, the depression in growth of the rye based upon the averages of transpiration and weight of the green tops, as just explained, is represented by 100 in each case, and the depression in the growth of barley in individual instances is ex-

[1] Contribution No. 247 from the Agricultural Experiment Station of the Rhode Island State College.

pressed as related thereto. In different experiments and under a number of different conditions the depressing effect on barley caused by the acid was, in comparison with 100 as representing the effect on rye, 124, 103, 102, 101, 95, 100, 99, 99, 96, 121, 98, 98, 96, 120, 75, 113; average, 103.

This agrees with the earlier work in showing that barley and rye seedlings are affected practically alike by acid added to a nutrient solution containing the usual essential elements.

Realizing that conditions attending the growth of seedlings in water culture are quite different from those in the field, a nearer approach to field conditions was made by growing the seedlings in sand and nutrients with and without the addition of acid, usually sulfuric. Sometimes sea-beach sand and again quite pure quartz sand was used without any special treatment. In other experiments the sand was digested in hot acid, washed, and ignited previous to its use. If the depression in the growth of rye is expressed in each case by 10, the relative depression in the growth of barley becomes 18, 8, 23, 11, 22, 9, 13, 6, 9, 10, 10, 13, 10, 13, 14, 4, 12, 17, 25, 18, 26, 10, 12, 15, 21, 27, 16, 8, 17, 10, 10, 23, 12, 6, 13, 4, 12, 13, 14, 13, 10, 15, 13, 11, 16; average, 14.

It may be seen that on the average the sulfuric acid depressed the growth of barley more than it did that of rye. In many instances, however, the difference was slight, as was nearly always the case in water cultures, and the authors were inclined to the opinion at the time that the exceptions were accountable mainly to abnormal differences in the relative vigor of the two kinds of seedlings. For the purpose of determining the difference in the effect of acid on various strains and varieties of barley and rye, a number of samples of these grains were kindly furnished upon request, by the agricultural experiment stations of the states of Utah, Minnesota, Iowa and Pennsylvania, and of the province of Ontario.

It was found by carrying on water cultures with these seeds that when the various strains were grown under the same conditions, either in neutral or acid cultures, there were some marked differences in the relative results. Therefore, in order to ascertain whether the relative effect of acid on the two kinds of seedlings was really dependent on the method of culture, water cultures and prepared sand cultures were carried on at the same time and with the same lot of seeds in each of a number of tests. The results are given in table 1, where it may be seen that the relative depression was about the same with the two kinds of seedlings, both in the water and sand cultures.

These results show that acidity present in connection with the ordinary plant nutrients has a relative effect on the two kinds of seedlings very different from that of so-called acidity in connection with the soil; for, as has been shown in the field repeatedly at this station, barley is much more sensitive than rye to acid-soil conditions.

TABLE 1

The relative effect of acidity on the weight (in grams) of the green tops of barley (B) and rye (R) seedlings when grown in sand and water cultures

	AUGUST 1911		SEPTEMBER 1911		MARCH 1913		MARCH 1913		MARCH 1913		RELATIVE AVERAGE WEIGHT	
	B	R	B	R	B	R	B	R	B	R	B	R
Sand culture												
Nutrients	4.5	3.6	3.9	2.6	4.2	1.9	4.4	2.2	3.7	2.2	100	100
	4.3	3.6	4.2	2.5	4.0	1.9	4.5	2.2	3.8	2.1		
Nutrients plus least amount of acid	3.7	2.8	3.5	2.2	2.9	1.5	3.4	1.8	3.3	1.6	82	81
	3.4	2.8	4.0	2.3	3.0	1.5	3.7	1.9	3.1	1.2		
Nutrients plus largest amount of acid	1.4	2.2	3.1	1.5	1.0	0.8	2.2	1.1	1.6	1.1	47	59
	1.3	1.8	2.7	2.0	1.1	1.0	2.8	1.3	2.3	1.5		
Water cultures												
Nutrients	5.3	5.6	8.1	4.5	8.4	6.2	8.2	5.6	8.8	6.7	100	100
	5.1	5.8	7.9	4.0	8.8	6.2	8.2	4.9	8.9	5.9		
	5.2	6.2	8.3	4.3								
Nutrients plus least amount of acid	3.3	4.7	7.1	4.2	8.3	4.3	7.4	4.1	7.7	5.6	88	84
	3.6	5.5	7.8	4.3	8.1	4.3	7.6	4.1	7.9	5.2		
	4.2	5.7	7.4	3.5								
Nutrients plus largest amount of acid	2.1	3.7									54	60
	2.5	3.5			7.1	2.9	6.4	3.1	6.5	4.1		
		3.5			6.9	2.7	5.7	2.9	6.0	3.6		

INFLUENCE OF THE EXTRACT OF AN ACID SOIL

That the special characteristics of an acid soil are transmitted to its extract is shown by the effect of an alkaline material, sometimes the hydroxide of sodium and again of calcium, either on the aqueous extract of soil from permanent plat 23 or on the same extract after adding nutrients. For a number of years this unlimed soil had received sulfate of ammonia, as the source of nitrogen in fertilizer chemicals. It was therefore very acid and imparted sufficient of its characteristics to the extract so that barley seedlings grew much less normally than rye seedlings, unless alkaline materials especially were added to the extract. The following numbers represent the percentage loss in weight of rye and barley seedlings caused by withholding alkaline materials from, in comparison with adding them to, the extracts of this soil:

Rye.....−2, 2, 22, 7, −10, 30, 49, 16, 36, 1; Average, 15
Barley..... 41, 36, 44, 39, 10, 61, 57, 52, 64, 49; Average, 45

When it had been shown that rye was much less affected than barley by acid soil and its aqueous extract, whereas acidity produced artificially in sand and water cultures affected both seedlings about alike, the question arose as to whether the soil and extract contained some factor which protected the rye from the usual deleterious effect of acidity as exhibited in artificial cultures. Any treatment which would destroy such a possible agency for protecting the rye seedlings would be expected to exert a relatively greater detrimental influence on the rye than on the barley which appears not to be protected. To throw light on this point possibly, seedlings were grown in beakers and the effects noted of certain soil treatments which will now be mentioned.

Under like manurial conditions barley and rye seedlings were grown (a) in unheated soil from the unlimed sulfate of ammonia plat, (b) in the same soil after sterilization in an autoclave at about 125°C. under 15 pounds pressure for one hour, and (c) in similarly sterilized soil to which an infusion from the original soil was added subsequently. The two experiments which were

TABLE 2

The effect on the growth of barley and rye seedlings of heating an acid soil at different temperatures

	EXPERIMENT I RELATIVE WEIGHT OF GREEN TOPS		EXPERIMENT II RELATIVE WEIGHT OF GREEN TOPS	
	Barley	Rye	Barley	Rye
Unheated soil	100	100	100	100
Soil heated at 100°C	82	94		
Soil heated at 260°C	36	36	53	45
Soil heated at 360°C	113	94	116	90
Soil heated at 420°C			121	100

conducted did not furnish evidence that the hypothetical agent for protecting the rye consisted of some organism, for the soil infusion was not more beneficial to the rye than to the barley. The barley especially, however, made a very unsatisfactory growth due to the acid-soil conditions. From a similar viewpoint the soil was treated with toluol instead of being sterilized by heat, but the results do not warrant any statement of interest in connection with the idea that the rye was influenced by the association of some lower form of life which enabled it to withstand the acid conditions. Coville (2) claims that the blueberry, another acid-soil plant, has a mycorrhizal fungus associated with it "which is able to assimilate nitrogen from the surrounding organic matter, and perhaps from the atmosphere also, and to convey it into the plant without taking along with it a large amount of the poisonous soil moisture."

Soil taken from the unlimed ammonium sulfate plat in the fall of 1913 was heated at various temperatures prior to growing rye and barley seedlings in it with optimum nutrients (table 2). By the calcium-acetate method, the

amount of calcium oxide required to neutralize two million pounds of soil was 7200 pounds for the unheated soil, and for soil heated several hours at 100°, 260° and 360°C., it was 5760, 5400 and 3240 pounds, respectively. When heated for 10 hours at not less than 420°C. the amount of calcium oxide required was reduced to 2200 pounds.

Although heating the soil decreased the acidity, there was a decided increase in toxicity when the temperature of heating was around 260°C., which, however, affected the two kinds of seedlings about alike. Heating at the higher temperatures developed no toxicity, but seemed to be beneficial for barley; indicating that some substance toxic especially to barley may have had its effect reduced by the heating. In view of evidence to be presented that aluminum is such a substance, the thought arises that its availability might have been decreased by dehydration effected by the heat.

ADDITION OF SUBSTANCES TO ASCERTAIN IF THEY HAVE AN UNLIKE EFFECT ON THE TWO KINDS OF SEEDLINGS

Inasmuch as rye and barley are affected differently by acid soils, it was evident that some factor besides acidity must be potent. It seemed desirable, therefore, to make next the following tentative tests to ascertain whether treatment with other materials than those of an alkaline nature would have a specific effect on either kind of seedlings.

The acidity of hydrogen peroxide was nearly neutralized with sodium hydroxide; and, after the nutrients had been supplied, two amounts, 3 and 5 cc., were added to 250 cc. of soil extract of plat 23. The smaller addition of hydrogen peroxide increased the transpiration, but otherwise the barley seedlings were more abnormal with both amounts of hydrogen peroxide than when it was not added to the soil extract.

One of the poisonous organic substances which have been isolated from soil by the United States Bureau of Soils is dihydroxystearic acid. The Bureau kindly furnished a small amount of this material so that it might be ascertained whether its relative effect on barley and rye seedlings was similar to that exerted by acid soil. One hundred parts of the dihydroxystearic acid per million parts of a nutrient solution depressed the transpiration of barley 77 per cent and of rye 85 per cent; likewise, the weight of green barley tops was reduced 40 per cent and of rye tops 54 per cent. Fifty parts per million depressed the transpiration of barley and rye, 47 and 49 per cent, respectively, and the weight of green tops, 30 and 24 per cent, respectively. In this last experiment an amount of sulfuric acid equivalent to the dihydroxystearic acid depressed the transpiration of the barley 48 per cent and the weight of its green tops, 12 per cent. In another similar comparison with barley, the dihydroxystearic acid depressed the transpiration 47 per cent and the weight 28 per cent, whereas the sulfuric acid depressed the transpiration 29 per cent and the weight 13 per cent.

It appears from the foregoing data that dihydroxystearic acid does not account for the fact that barley is more injured than rye by acid-soil conditions, since it was fully as deleterious to rye as to barley. It likewise appears probable that the toxicity of dihydroxystearic acid is largely attributable to its acidity.

The relative effect of manure extract when added to an acidulated nutrient solution was tested. The manure extract was acidulated, boiled, and then neutralized before using. The development of both kinds of seedlings was depressed 56 per cent by the addition of acid to the nutrient solution and only about 42 per cent when the manure extract was also added. The barley and rye seedlings were again affected alike, however, and no evidence was obtained, therefore, that the manure extract contained any organic compound which served to account for the difference in the effect of acid soils on the two kinds of seedlings.

A SEARCH FOR SOME DIFFERENTIATING FACTOR IN THE SOIL EXTRACT

Half of a soil aqueous extract, with 0.0015/N acidity, was distilled from a glass retort, with the result that the distillate proved to be neutral, and the acidity of the solution remaining in the retort was double that of the original. Barley seedlings were grown with optimum nutrients dissolved in the various liquids mentioned below and the following relative averages of the transpiration and weight of the green tops secured, namely: carbon-treated distilled water, 100; soil extract, 37; distillate from the soil extract, 108; and the soil extract from which a half had been distilled, 27. The transpiration in the last instance was only a half of what it was in the original soil extract, showing that the deleterious substance simply had been concentrated in the retort and not distilled off.

The toxic factors in the extract of the soil from the unlimed sulfate of ammonia plat were not segregated by dialysis, for after a strongly toxic extract had been placed in a dialyzer for one week, the diffusate and dialyzate were shown to be equally toxic to barley seedlings. This indicated that the toxicity was of a crystalloidal nature.

Upon failure to get evidence that the toxicity was probably due mainly to organic material, it was decided to continue the investigation more especially with inorganic substances in mind.

Since nitrification would not be expected to take place normally in the unlimed plat to which sulfate of ammonia is applied as a source of nitrogen, experiments were conducted with seedlings in solution to see if rye makes relatively better use than barley of sulfate of ammonia as a source of nitrogen. If such were the case, the ability of rye to grow better than barley on the plat might be explained. It may be seen from table 3, however, that the growth of the two seedlings was depressed about alike when calcium nitrate was replaced by ammonium sulfate on the basis of equal nitrogen, that is, an

average depression of 63 per cent. This depression was doubtless due to the development of acidity where ammonium sulfate was used, rather than to this particular nitrogen combination; for, as may be seen in the table, it was necessary only to have present with the ammonium sulfate some calcium carbonate or sodium hydroxide in order to obtain practically normal growth. It would hardly be expected that under the experimental conditions there would be nitrification, and it is true that at least in some of the solutions, ammonium salts were left as such after the removal of the seedlings. The residual solutions when made up to the original volume had an average alkalinity in case calcium nitrate was used of 0.0002/N with phenolphthalein, and of 0.014/N with methyl orange as indicators; whereas when ammonium sulfate was used, instead of alkalinity there was an average acidity of 0.019/N with phenolphthalein and 0.0008/N with methyl orange. Carbon dioxide was eliminated from the solutions before using phenolphthalein as an indicator.

TABLE 3

Relative effect of equivalent amounts of calcium nitrate and ammonium sulfate in nutrient solutions on the average of the relative transpiration and weight of green barley and rye seedlings

	EXPERIMENT							
	I	II	III		IV		V	
	Rye	Rye	Barley	Rye	Barley	Rye	Barley	Rye
$Ca(NO_3)_2$	100	100	100	100	100	100	100	100
$Ca(NO_3)_2$ plus $CaCO_3$							97	95
$(NH_4)_2SO_4$	44	38	31	43	32	35	35	35
$(NH_4)_2SO_4$ plus $CaCO_3$							91	87
$(NH_4)_2SO_4$ plus NaOH (varying amounts)		54			60	55	88	78

The acidity of the solutions when sulfate of ammonia was unaccompanied by a base was sufficient to account for the depression in the growth of the seedlings.

In order to ascertain whether the ash constituents of the soil extract were in any way responsible for its different effects on rye and barley, an aqueous extract was made, and evaporated to dryness. The residue was ignited, and then dissolved in hydrochloric acid. Most of the acid was evaporated, and the remainder neutralized with sodium hydroxide. Nitrogen was supplied in the solution in such a proportion of ammonium and calcium nitrates as was necessary to control the reaction of the solution. To a portion of the soil extract, which was not evaporated, the same amounts of nitrogen and of sodium chloride were added as were present in the other solution.

Barley and rye seedlings were grown under neutral and acid conditions, not only in the above solutions, but in a regular nutrient solution. The results may be found in table 4. The results are so arranged that the development

in the neutral solutions in each of the three cases is represented by 100 so that the relative effect of the acid cultures may be seen readily by referring to the table.

Rye exhibits its usual tendency, though less than has been sometimes the case, to withstand better than barley, the toxic effect of the soil extract in its original condition. It is of importance to notice that the same tendency also exists in connection with the acid nutrient solution containing the ash constituents only of the soil extract. That is, the growth of the rye was depressed much less than that of the barley. When the acid was added to the ordinary nutrient solution, however, the growth of the rye was, in general, depressed fully as much as that of the barley; an observation which is in accord with what has usually been noticed heretofore.

TABLE 4

The relative effect on barley and rye seedlings of an extract of an acid soil, of the ash in the same, and of an acid nulrient solution

	EXPERIMENT I				EXPERIMENT II			
	Relative weight of green tops		Relative transpiration		Relative weight of green tops		Relative transpiration	
	Barley	Rye	Barley	Rye	Barley	Rye	Barley	Rye
Soil extract of plat 23 neutralized with NaOH	100	100	100	100	100	100	100	100
Soil extract of plat 23 unneutralized	75	74	61	66	78	92	43	67
Solution of the soil-extract ash	100	100	100	100	100	100	100	100
Solution of the soil-extract ash plus H_2SO_4	82	107	75	94	74	86	43	54
Nutrient solution	100	100	100	100	100	100	100	100
Nutrient solution plus the H_2SO_4	74	79	56	58	59	50	40	25

Evidence having been secured that some inorganic element or elements were responsible for the different effects produced by the acid-soil extract, determinations were made in the fall of 1913 of certain ingredients in an aqueous extract prepared as follows from soil of the unlimed ammonium sulfate plat. Various 2-pound portions of the soil were each mixed with 2.5 liters of distilled water, and allowed to stand about an hour, after which the liquid parts were poured off to furnish 20 liters of extract, which was then passed through a Chamberland filter. This solution contained the following in parts per million: N, 15.9; SiO_2, 0.7; SO_3, 65.2, and Al_2O_3, 27.8. The last two were present in about equivalent amounts.

The two kinds of seedlings were next grown in the presence of certain of the elements not generally considered as exerting any nutrient influence on plant growth.

The effect of even chromium was determined, because a positive qualitative test for this element was reported upon examining the aqueous extract. Potassium chromium sulfate in an acid nutrient solution failed, however, to give indications that it inhibited the growth of rye seedlings any less than that of barley seedlings.

Although manganese was absent from the soil extract, recent work at the Alabama station (3) makes it of especial interest to record that in connection with three trials of potassium permanganate as an oxidizer, the results given in table 5 had been obtained, which showed that the effect of manganese on the two seedlings was not essentially different. The basal solution contained optimum nutrients.

A trace of iron was present in the soil extract but the authors had already failed to find that this element in acid solutions affected the two seedlings differently (6).

TABLE 5

Relative average transpiration and weights of green tops showing effect of potassium permanganate

	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		AVERAGE	
	Barley	Rye	Barley	Rye	Barley	Rye	Barley	Rye
No $K_2Mn_2O_8$	100	100	100	100	100	100	100	100
2 parts per million $K_2Mn_2O_8$	91	86	84	95			87	91
4 parts per million $K_2Mn_2O_8$	87	78	89	97	90	88	89	88
8 parts per million $K_2Mn_2O_8$	84	71			81	73	82	72

ALUMINUM AS THE CAUSE OF THE UNLIKE EFFECT OF ACID SOIL ON RYE AND BARLEY SEEDLINGS

The relative percentage depression in the average of the transpiration and weight of green rye and barley seedlings caused by adding to a nutrient solution sufficient aluminum, usually in sulfate, to produce in most cases about the same concentration of aluminum as was present in the soil extract, is shown below by different comparative tests:

Rye.....31, 30, 30, 26, 18, 18, 17, 30, 21, 34, 37, 22, 15, 19; Average, 25
Barley.....67, 66, 62, 47, 47, 68, 62, 57, 44, 57, 46, 56, 48, 42; Average, 55

It may be seen from the preceding values that the aluminum and accompanying acidity are much less deleterious to rye than to barley; whereas it has been shown previously that the two seedlings are affected alike by acidity when unaccompanied by aluminum. In most instances the two sets of conditions, with and without aluminum, existed in the same experiment so that the differences could not be attributable to other varying conditions. Similar results were obtained also in sand cultures.

It having been shown that there exists a specific difference in the effect of aluminum on rye and barley, it seemed probable that the element either tends

to protect rye more than barley from the deleterious effect of acidity, or else it is less toxic to rye than to barley. In either case the growth of the barley would be depressed more than that of the rye.

The possibility was recognized that the association of aluminum with chromium or with silicon, two other non-nutrient ingredients of the aqueous extract, might be even more effective than the aluminum itself. A number of water cultures containing in the nutrient solution different proportions of these elements were conducted, but it was found that chromium and silicon, either with or without aluminum, exerted no unlike effect upon the two seedlings.

To decide between the two hypotheses regarding the manner in which aluminum affects rye and barley differently, it was necessary to know the comparative acidity of the nutrient solution when, to one aliquot, aluminum sulfate was added, and to another, an equivalent amount of free sulfuric acid. Under these two conditions the barley was depressed equally and the rye very unequally.

If the hydrolysis of the aluminum sulfate was insufficient to produce a degree of acidity about like that of the free sulfuric acid, then the depression in the growth of the barley must be attributable in part to the toxicity of the aluminum. Miyake (7) found that the hydrogen-ion concentration of a solution of aluminum chloride was about one-third as great as in an equivalent solution of hydrochloric acid. This led him to think that aluminum itself in some form might have been toxic, since the above-mentioned solutions with which he worked proved to be about equally injurious to rice seedlings.

In our work the nutrient solution containing aluminum sulfate was found to have about one-fourth the hydrogen-ion concentration which existed when an equivalent amount of sulfuric acid was added. This led the authors to believe that aluminum exerted its different effect because of its more pronounced toxicity to barley rather than because of an exclusive protective influence on rye.

It was recognized that extreme care was necessary in working with nutrient solutions containing such elements as phosphorus, iron and aluminum to be sure that the effect of the aluminum was not prevented by precipitation. Had this occurred in the rye cultures more than in the barley cultures, for instance, the conclusion that aluminum was less toxic to rye than to barley would have been erroneous. Owing to the difficulty of differentiating with certainty in culture solutions between a slight chemical precipitation, and turbidity naturally arising from other suspended material, certain precautions are necessary in work of this kind. Furthermore, without special precautions precipitation might take place on the roots.

When the phosphorus was reduced to only the very small amount necessary for nutrient purposes, and such proportion of ammonium nitrate and calcium nitrate used as would maintain or increase the acidity, it was felt that if a solution remained perfectly clear from standing prior to the intro-

duction of the seedlings, it was not probable that any subsequent precipitation could have taken place.

Some attempt was made to ascertain whether aluminum is toxic in neutral or alkaline solutions, using citric acid and tartaric acids or their sodium salts for the purpose of holding the aluminum in solution, even if in complex ions. Under these conditions, however, the two seedlings seemed to be affected more nearly alike, and the difficulties of knowing positively whether the aluminum had been maintained in true solution led to the abandonment of this line of work at least temporarily. It would be very desirable to know the range of circumstances in which aluminum is toxic.

TABLE 6

The relative effect of aluminum sulfate, and its equivalent and twice this amount of sulfuric acid, on the weight of the green tops per ten barley and ten rye seedlings

SPECIAL ADDITIONS TO THE NUTRIENT SOLUTION	WEIGHT OF GREEN TOPS			RELATIVE WEIGHT OF GREEN TOPS		
	Barley (2-rowed)	Barley (6-rowed)	Rye	Barley (2-rowed)	Barley (6-rowed)	Rye
	grams	*grams*	*grams*			
None	3.88	4.63	2.78	100	100	100
	3.80	4.63	3.09			
8 cc. 0.1/N sulfuric acid	2.12	3.05	1.73	58	59	59
	2.26	2.26	1.60			
4 cc. 0.1/N sulfuric acid	3.29	3.73	2.11	82	76	83
	2.86	3.23	2.60			
Aluminum sulfate (added sometime previously)		3.32	2.43		67	86
		2.85	2.54			
Aluminum sulfate (just previously added)	2.59	2.91	2.09	68	65	83
	2.51	3.04	2.72			

An experiment typical of the many which have been conducted with aluminum salts will now be described somewhat in detail. It was conducted in the greenhouse between January 8 and 29, 1918. The results may be found in table 6. In the series where the aluminum sulfate is there mentioned as having been "added sometime previously," it was mixed with the nutrient solution a few weeks before the experiment was begun, to afford full opportunity for the aluminum to be precipitated if any of the ingredients could throw it out of solution. In the other series the aluminum sulfate was not added to the nutrient solution until just before it was supplied to the seedlings. There was no significant difference, however, in the two series.

Even if the aluminum remained in solution prior to the introduction of the seedlings, it was recognized that the nutrient solution must not become physiologically alkaline as a result of the growth of the seedlings for fear that the aluminum would be precipitated as a consequence and the purpose of the experiment be thwarted. Previous tests had taught us that the reaction of the nutrient solution may be controlled conveniently by varying the ratio of calcium nitrate to ammonium nitrate, and in the present experiment a sufficient proportion of ammonium nitrate was added so that the acidity of the solutions would at least not be reduced in the presence of the growing seedlings.

A liter of the basal nutrient solution contained 15 cc. 0.2/N $Ca(NO_3)_2.4\,H_2O$, 10 cc. 0.1/N NH_4NO_3, 8 cc. 0.1/N KCl, 8 cc. 0.2/N $MgSO_4.7\,H_2O$, 1 cc. of a solution containing 8.3 gm. of $CaH_4(PO_4)_2.H_2O$ per liter, and 10 cc. of a dilute $Fe_2(NO_3)_3$ solution. In the series receiving the larger addition of sulfuric acid 8 cc. of a 0.1/N solution was also added in the liter. The aluminum sulfate was made equivalent to this larger application of acid, and it may be seen that the aluminum depressed the growth of barley much more than it did that of rye, although again both seedlings were depressed alike by either concentration of acid.

TABLE 7

Percentage depression caused by sulfuric acid and by aluminum sulfate

TRANSPIRATION				WEIGHT OF GREEN TOPS			
Barley		Rye		Barley		Rye	
H_2SO_4	$Al_2(SO_4)_3$	H_2SO_4	$Al_2(SO_4)_3$	H_2SO_4	$Al_2(SO_4)_3$	H_2SO_4	$Al_2(SO_4)_3$
65	71	64	31	40	43	42	28
61	54	59	18	38	33	41	24
58	58	62	31	48	56	49	37
62	49	53	34	59	42	47	40
Average 62	58	60	29	46	44	45	32

Although it is obvious that the growth of barley is depressed much more than that of rye by aluminum, it is of interest to consider whether the rye is actually affected by the aluminum itself. The results given in table 7 will assist in consideration of this point. As there arranged, the percentage depression is given on both the transpiration and weight of the green tops caused in four typical experiments by the addition singly of sulfuric acid and aluminum sulfate in equivalent amounts to an optimum nutrient solution.

It may be seen that barley was affected about alike by equivalent amounts of sulfuric acid and aluminum sulfate, but that rye was depressed about twice as much by the acid as by the aluminum salt. If the latter is hydrolyzed about one-fourth as extensively as the acid, which seems to be the

case, judging from the hydrogen-ion determinations referred to previously, then not more than half the depressing effect exerted on the rye would seem to be attributable to acidity, leaving a portion of the effect to be chargeable directly to the aluminum even in case of the rye. From this point of view aluminum would seem to be some three times as toxic to barley as to rye. Only an approximate quantitative consideration, however, is warranted until more work has been done on the actual acidity of the nutrient solution as influenced by the addition of the sulfuric acid and aluminum sulfate, both before and after the growth of the barley and rye seedlings. Judging from the appearance of the roots of the seedlings, there was very little effect of the aluminum on the rye but a very marked effect on the barley (fig. 2, plate 1).

Such experiments seem to warrant the idea that aluminum itself in solution in certain acid soils may be a factor differentiating their effect on plant genera, whereas acidity from whatever source may affect the plant genera alike. Ruprecht and Morse (8) at the Massachusetts station did not discriminate as to which component of the aluminum salts was responsible for toxicity.

Investigators at the Indiana station state in connection with their work (1, p. 368) as follows:

> The facts that aluminum nitrate and soil extract of the same normality with respect to aluminum are of approximately equal toxicity, and that aluminum nitrate and nitric acid of the same normality are of approximately equal toxicity, point to the acid as the toxic agent.

If in our search for a cause of the different effect of acid-soil conditions on different kinds of plants we had not rigidly excluded every toxic factor which affected alike the two kinds of plants, which were selected because of the unlike effect of acid soils upon them, it is doubtful if a probable cause would have been discovered.

One could find readily enough, many factors toxic to plants in general. An investigator is liable to fix his attention on one of these, and because of circumstantial evidence which he accumulates, conclude that he has found the cause which is responsible for the deleterious effect of acid soils.

If, however, the very genera which are affected decidedly differently by a particular set of acid-soil conditions are grown in circumstances where a specific factor is the only variant, it will be found that many of the assumed causes are only contributory to the principal reason for the effects observed in the field.

It may be found eventually that the principal disturbing factor is different under one set of acid-soil conditions than under another set, and even that the same factor may not account for the different effects in case of all genera.

EFFECT OF MANURIAL TREATMENTS OF THE SOIL ON THE AMOUNT OF ACTIVE ALUMINUM

Inasmuch as aluminum is toxic, in connection with acid, to barley and probably other crop plants, it may be true that the improvement of certain soils by liming or phosphating is due in part to a reduction in the amount of active aluminum. To gain indications regarding this point, in the spring of 1914 samples of soil from certain permanent field plats were taken, dried, and sifted through a 1-mm. sieve. With frequent shaking for a period of three days, the soil was treated with water through which carbon dioxide was conducted in such a way as to maintain a saturated solution and atmosphere.

The following determinations were made:

PLAT NUMBER	SPECIAL FIELD TREATMENT	Fe_2O_3, Al_2O_3, P_2O_5	Fe_2O_3	Al_2O_3, P_2O_5
		per cent	*per cent*	*per cent*
23	Sulfate of ammonia, unlimed	0.063	0.007	0.056
25	Sulfate of ammonia, limed	0.040	0.010	0.030
27	Nitrate of soda, unlimed	0.044	0.010	0.034
29	Nitrate of soda, limed	0.036	0.010	0.026
54	Dissolved bone, unlimed	0.059	0.007	0.052
66	Roasted aluminum phosphate, unlimed	0.047	0.007	0.040
68	No phosphate, unlimed	0.080	0.008	0.072
67	No phosphate, limed	0.048	0.014	0.034

The amount of aluminum and phosphorus oxides, which was determined by difference, proved to be too small for separation of the two. The first four plats received an equal liberal amount of acid phosphate annually. However, experiments at this station have shown that liming tends to increase the availability of phosphorus; consequently, if the differences are attributed to aluminum there will be at least no exaggeration. In no case had the liming been sufficient to maintain even a neutral soil reaction. Nevertheless, the results show that there was probably more active aluminum in the more acid soils, especially the very acid soil from plat 23. The four remaining plats are a part of the "phosphate experiment." The results suggest that both the phosphating and the liming may have reduced the active aluminum, even though here again the soil has never been fully neutralized. The data are recorded mainly for their suggestiveness, with a full realization of their incompleteness.

To ascertain the influence of uncombined phosphoric oxide in reducing the amount of active aluminum in soil from the unlimed sulfate of ammonia plat, 250-gm. lots of the soil were mixed intimately with water and different amounts of a solution, 25 cc. of which contained 2.5 gm. of P_2O_5, and allowed to stand for about a month, when the following was found to be soluble in 20 per cent acetic acid by digesting for 24 hours at 50 to 60°C. with occasional shaking:

	P_2O_5	Al_2O_3*
	per cent	*per cent*
Check soil receiving no P_2O_5	0.013	0.566
Soil receiving 1.3 gm. P_2O_5	0.015	0.524
Soil receiving 1.9 gm. P_2O_5	0.046	0.260
Soil receiving 3.0 gm. P_2O_5	0.077	0.101

* The following percentages of certain ingredients sometimes claimed to be in a deleterious form have been found from time to time in the dry fine soil of the same general nature, which is a granitic glacial drift, namely: Al_2O_3 4.64, Fe_2O_3 3.46, Mn_3O_4 trace, soluble in a hot, strong solution of hydrochloric acid; Al_2O_3 and Fe_2O_3 1.46, soluble in 0.20/N HNO_3 in 5 hours at 40°C.; Al_2O_3 and Fe_2O_3 0.50 ,soluble in 0.04/N HNO_3; and Al_2O_3 0.20, soluble in 0.013/N HNO_3. Not enough iron was dissolved in the latter solvent to impart a yellow tint to the aluminum hydroxide precipitate, showing how relatively soluble the aluminum was in very dilute acid. Not more than a tenth as much calcium and magnesium oxides as aluminum and iron oxides is dissolved by the various solvents.

Indications were likewise obtained as to the effect of acid phosphate upon both the reaction and the aluminum of an acid soil, by using a sample which was taken from the unlimed sulfate of ammonia plat early in the spring of 1914. Three lots of soil of 2,000 gm. each were mixed respectively with 95, 190 and 380 gm. of acid phosphate. They were then kept moist and stirred frequently for about three weeks, when it was found by the calcium-acetate method that the calcium oxide requirement in pounds per two million pounds of soil was as follows: check soil, 4608; smallest application of phosphorus, 5472; medium, 7272; and largest, 11,880. The same soil treated in a similar manner, except that the lots stood for six months instead of three weeks, exhibited by the ammonia method[2] a similar increase in lime requirements with the increasing phosphate applications: namely, 7194, 9184, 11,463 and 14,783 pounds. Then the different lots of soil were placed in beakers, and nutrients were added, including a small application of mono-calcium phosphate to the soil untreated with the acid phosphate. Calcium carbonate was also added in certain beakers. Barley and rye seedlings were then grown with the average results shown in table 8, which as usual are the averages of duplicate cultures.

As usual the rye grew much better than the barley on the untreated acid soil, but not after the liming. Even the smaller amount of acid phosphate represented an application of nearly 50 tons per acre; that is, it was much larger than is made in farm practice. It is important to notice that in spite of the large amount of acidity represented by this application, the growth of the barley was markedly increased and the amount of active aluminum decreased. This reduction in active aluminum was not accompanied by an

[2] Submitted by the late L. P. Howard of the Rhode Island station to the 1916 Convention of the Association of Official Agricultural Chemists for publication in its journal, but not yet printed.

increased growth of rye, thus furnishing further evidence that aluminum is much less toxic to rye than to barley.

The progressive toxicity of aluminum sulfate was demonstrated by adding to 250 gm. of an unlimed fertilized soil in different beakers, 4, 8, 12, 16 and 24 cc. of a solution containing 13.665 gm. of hydrated aluminum sulfate per liter, and then growing barley. Without the aluminum, 1.90 gm. of the green tops were produced, and with the successive aluminum applications, 1.88, 1.30, 1.30, 1.15 and 0.85 gm., respectively.

Characteristic growth responses by barley and rye were observed by planting their seeds with basal nutrients in an unlimed soil from permanent plat 84, which for years had received no manurial treatment in the field. Without special treatment barley, as usual on acid soil, made a poor growth, 39 gm. of dry tops per 8-inch pot; while rye produced 60 gm., or the same as the barley after the soil was limed. The addition of 4.5 gm. of hydrated aluminum sulfate to the unlimed soil depressed the growth of barley 32 per cent and that of rye 11 per cent. An equivalent amount of sulfuric acid depressed

TABLE 8

Results showing effect of acid phosphate on the amount of active aluminum in the soil

SPECIAL SOIL TREATMENT	WEIGHT OF GREEN TOPS		Al_2O_3 SOLUBLE IN DILUTED ACETIC ACID
	Barley	Rye	
	gm.	*gm.*	*per cent*
None	1.05	2.02	0.105
Calcium carbonate	2.33	1.94	
Smaller amount of acid phosphate	1.80	1.74	0.039
Calcium carbonate and larger amount of acid phosphate	1.95	1.95	0.005

the barley 40 per cent and the rye 11 per cent. Again the aluminum depressed the barley more than the rye. Probably the added sulfuric acid associated itself with sufficient aluminum in the soil so that its action was similar to that of the aluminum sulfate, for by itself the acid has been shown repeatedly to depress both kinds of plants alike.

To obtain on a larger scale the effect of thorough phosphating on plants which usually respond favorably to liming, cultures were carried on in 8-inch Wagner pots, using soil from the unlimed sulfate of ammonia plat. Four different experiments were conducted using the ordinary nutrients, including phosphorus for nutrient purposes, in each pot. When this was the only treatment both table beets and cos lettuce made practically no growth and the less sensitive barley only a small one. In the first experiment with beets, the maximum yield of roots, brought about by liming was 350 gm. per pot, obtained with about 3800 pounds of calcium oxide per acre in finely divided hydrate. In spite of treatment with probably too large an amount of acid phosphate for beets, even for the purpose at hand, at the rate of about 50

tons per acre, there was a production of 167 gm. per pot, although the acid reaction to litmus paper has been much intensified. In the second similar experiment with beets it was possible to produce only 200 gm. by optimum liming; while phosphating alone, with 14 tons of acid phosphate per acre, led to the production of 107 gm. In the third experiment 200 gm. of green lettuce leaves were produced with the optimum application of lime and 73 gm. where 14 tons of acid phosphate per acre was used. A phosphating with twice this amount, however, resulted in 272 gm., or much more than was produced by any other of a large number of treatments which were made for another purpose in the same experiment (fig. 1, plate 1). The results of the fourth experiment, with barley, are shown best by the yields of the dry, mature crop given in table 9.

The striking fact is that acid phosphate in abnormal applications, which increased the acidity of acid soils, should promote normal growth where practically no growth of very sensitive crops was possible before its application.

TABLE 9

Results showing the effect of acid phosphate on the yield of barley

	DRY BARLEY STRAW AND GRAIN PER POT	
	Grams	Relative weight
No special addition	36	100
Precipitated $CaCO_3$, 2.75 tons per acre	64	178
Acid phosphate 25 tons per acre	92	256
Acid phosphate 25 tons, and $CaCO_3$ 2.75 tons per acre	105	292
Acid phosphate 50 tons per acre	126	350
Acid phosphate 50 tons, and $CaCO_3$ 2.75 tons per acre	134	372

The practical advantages of phosphating and liming may often prove to be due to the precipitation of aluminum quite as much as to supplying phosphorus as a nutrient and lime as a reducer of acidity.

SUMMARY

In the present study of the cause of the unlike effect of acid soils on different kinds of plants, rye was chosen as a plant which will grow well on such soils, and barley as one which is quite deleteriously affected by the same conditions.

Seedlings of these two plants were, however, affected about alike by a given amount of acidity in connection with nutrient culture both in water and in sand.

Sterilization of the soil, either by heat or toluol, did not cause any change which with certainty influenced differently the two kinds of seedlings.

Substances which affected rye and barley about alike, and therefore did not suggest the real differentiating factor, were as follows: Hydrogen peroxide, dihydroxystearic acid, manure extract, ammonium sulfate, potassium permanganate, chromium and silicon.

The aqueous extract of an acid soil affected barley and rye the same as the acid soil itself. By distillation, the toxic principle of the extract was concentrated in the residue, the distillate being nontoxic. The dialyzate and diffusate arising from dialysis of the extract both had the same effect upon the two seedlings.

The search was made among the inorganic constituents for the active factor, largely because the ash of the soil extract when brought into solution had the same effect as the acid soil.

Aluminum proved to be the element which was responsible for the different influence on the plants.

Equivalent amounts of aluminum sulfate and of sulfuric acid, when added to an optimum nutrient solution, produced about the same depression on the growth of barley. Although the sulfuric acid caused a similar depression of the rye, the aluminum salt caused very little depression, and scarcely affected the rye roots. The nutrient solution when it contained the acid was found to have about four times the concentration of hydrogen-ions as when it contained aluminum sulfate. Therefore, the toxic effect of the latter on the barley is attributable largely to the aluminum.

Treatment of an acid soil with either phosphoric oxide or acid phosphate reduced the amount of active aluminum in the soil. Unusually large additions of acid phosphate caused remarkable growths of plants so sensitive to an untreated acid soil that previously no growth was possible, and this was in spite of the fact that the acidity of the soil was very much increased by the acid phosphate. The active aluminum, however, was much decreased by the treatment.

The results indicate that the practical advantage of phosphating and liming may often prove to be due to the precipitation of active aluminum quite as much as to supplying phosphorus as a nutrient and lime as a reducer of acidity.

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PLATE 1.

Fig. 1 (see p. 275). Cos lettuce, showing that an unusually large amount of acid phosphate was more beneficial than lime in case of an acid soil upon which lettuce could not grow. All pots received the same basal optimum nutrients.

	green lettuce gm.
1. (At left.) No special addition	0
2. 1500 lbs. per acre of CaO in hydrated lime	70
3. 2000 lbs. per acre of CaO in hydrated lime	145
4. 3000 lbs. per acre of CaO in hydrated lime	179
5. 4000 lbs. per acre of CaO in hydrated lime	201
6. 28 tons per acre of acid phosphate	277

Fig. 2 (see p. 271). Showing the like effect of acid and the unlike effect of an equivalent amount of aluminum sulfate on barley and rye. All bottles received the same basal optimum nutrients.

	Relative transpiration	Relative weight of green tops
1. (At left.) Barley with no special addition	100	100
2. Barley with sulfuric acid	31	55
3. Barley with aluminum sulfate	24	45
4. Rye with no special addition	100	100
5. Rye with sulfuric acid	35	54
6. Rye with aluminum sulfate	65	75

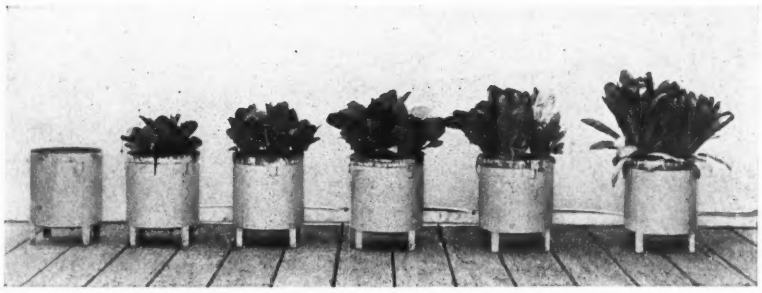

FIG. 1

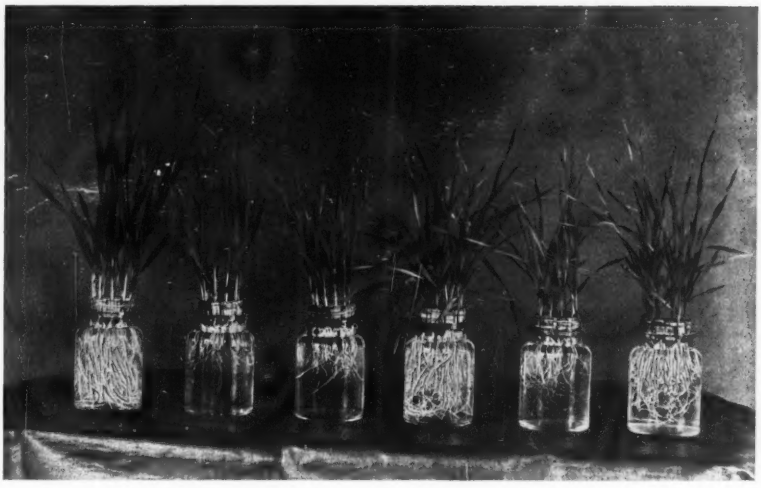

FIG. 2

THE MOVEMENT OF PLANT-FOOD WITHIN THE SOIL[1]

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Received for publication October 15, 1918

INTRODUCTION

The solubility of the several plant-food elements used in fertilizers and the power of the soil to fix these elements when they are used in a soluble form as fertilizers are subjects which have received considerable attention from investigators in the past, regarding which there has been conflicting opinions, and about which there are still many things unknown. This present work is an attempt to learn, if possible, by a study of some of the fields which have been longest under experimentation, what finally becomes of the fertility elements added to the soil as fertilizers when not completely removed from the land in crops.

It has long been known that nitrogen in the form of nitrates is very often found in drainage waters, that drainage from fertile soils contains relatively smaller amounts of potassium and ammonium, and that phosphorus, when found at all, appears only in traces.

In spite of these facts, it cannot be doubted that, when used as fertilizers, the most soluble are retained to some extent in the surface soil and the most readily and firmly fixed elements gradually undergo some change and are carried deeper into the soil or are taken up by plants in a soluble form and either removed from the land or returned, again to go through the same processes.

It is a fact that should be well taken into consideration that certain substances which are generally considered insoluble are, nevertheless, washed from the soil appreciably, if not completely, by the leaching action of percolating rain-water over geological periods. It is an established fact in geological science that the comparative age of soils receiving somewhat similar amounts of rainfall may be told by the amounts of limestone which they still contain. Hilgard, after comparing the analyses of about a thousand soils from arid and humid regions, reports (21, 22) that arid soils, as a rule, contain about three times as much potassium, and twelve to fourteen times as

[1] This paper was submitted in partial fulfilment of the requirements for the degree of Master of Science in Agronomy in the Graduate School of the University of Illinois.

[2] The writer is indebted to Dr. C. G. Hopkins for helpful suggestions and criticisms in regard to the experimental work and the preparation of the manuscript.

much lime as do humid soils, and also more magnesia. Although he reports (21) no material difference in the amount of phosphorus contained in arid and humid soils, he says that there is much less organic matter in the arid soils, and since phosphorus is quite closely associated with the organic matter of soils, as shown by Stewart (42), there must be more phosphorus in mineral combination in arid than in humid soils. Although Alway found (1) but little difference in fertility elements between the Loess soils in the eastern part of Nebraska, where the rainfall amounts to about 30 inches per year, and the Loess soils in the western part of Nebraska, where the rainfall amounts to about 20 inches per year, a comparison of the content of the different fertility elements in comparable types of soils in the different glaciations of Illinois (23, 24) shows that there is a decrease from the most recently formed soil to the soil of oldest formation. With but two exceptions in the analytical data presented, giving the composition of Illinois soils, the nitrogen content of the surface seven inches decreases from the newer to the next older formed soils down through the seven glaciated areas, from 6750 pounds per acre in the late Wisconsin glaciation to 2880 pounds per acre in the lower Illinois glaciation; the phosphorus content decreases gradually, with but one exception, from 1410 to 840 pounds; and the potassium, also with but one exception, decreases gradually from 45,020 to 24,940 pounds per acre. Had the limestone not all been washed out of these surface soils, it cannot be doubted that this, too, would have shown the same gradual decrease in amount, but, after limestone is gone, the degree of limestone requirement developed depends more, perhaps, upon the nature of the remaining soil constituents than upon the amount of leaching which the soil undergoes, and yet, between the newest and the oldest soils there is a difference of 1100 pounds per acre. The data here presented (23) show that the organic matter has decreased faster than nitrogen, phosphorus or potassium, and we must assume either that the organic matter remaining is richer in these elements or that, when they have been released by the decay of organic matter, they have been fixed, partially at least, by the mineral constituents of the soil. If, however, the older soils were once as rich in these fertility elements as the newer soils now are, and there seems to be no reason for doubting it, then we must also assume either that these elements have not been completely and permanently fixed or that the mineral soil constituents with which they have united have been mechanically lost from the soil. The latter explanation does not seem sufficient, because there is but little erosion on the prairie soils of which these are types. Thus it appears that the different plant-food elements when present in soluble form are, at first, fixed by the soil and afterwards gradually leached out and washed away to some extent. That this process of loss is very slow is most vividly seen in the fact that many soils, though ages old, still contain large amounts of plant-foods, as potassium for example, and with respect to certain elements, not enough becomes soluble during a growing season to supply the needs of a large crop. One factor which operates to make the loss of plant-food slower

is the growth of the plants themselves. As reported in the several county reports for Illinois and those from Iowa and Kentucky, the nitrogen, organic carbon and usually the phosphorus are higher in the surface soil than in lower depths, especially in the soils which are level and not subject to erosion. This is not always true of potassium and calcium, there being, very often, less of these elements in the surface than in the lower depths. It has been stated that plants send their roots into the subsurface and subsoil areas where they take up plant-foods, use them to build up aerial tissues and, in nature, when they die and decay, leave these plant-foods in the surface soil. This is, no doubt, true to some extent, but the greatest activity is in the surface. By far the greatest amount of feeding takes place in the first seven inches of the soil and regardless of whether or not appreciable amounts of plant-food are brought up from lower depths, the elements which, when they become soluble in the surface soil, are taken up by the plant and rendered incapable of being leached away, will be conserved in the surface, while other materials not taken up or not rendered incapable of being washed out more easily, as potassium when the plant ripens, will be lost faster. Thus the per cent of phosphorus, for example, may increase in the surface not only because of being brought up from below, but because what is already there is retained while other materials are being decreased.

Whether or not the several factors operate to prevent the loss of materials added to soils as fertilizers, keeping them in the area of greatest activity and the best feeding range of crops, where they may become soluble or may be made available to these crops, is of prime importance to the method of fertilization. If the materials added as fertilizers are lost from the feeding zone of plants, then excesses should not be applied. On the other hand, if they remain in the feeding zone and become as insoluble as the elements placed there by nature, then why should not these elements be applied in the natural form and the effort made to bring about conditions in the soil such that they will become available to plants fast enough to insure good crops?

LITERATURE CITED

In order to get a comprehensive idea of the work done by other investigators which bears upon this subject, one must examine the results of experiments conducted in many ways with other objects in view than the determination of plant-food movement. Some of these will be simply drainage-water analyses, some will be irrigation studies, some will be a study of the chemical compounds found in the soil, and there are still many others. Such comment as is made upon the literature is divided somewhat into that dealing with the subject in a general way and that dealing primarily with each of the elements, phosphorus, nitrogen, potassium, calcium, magnesium and limestone.

Literature of the subject in general

Warrington, after a study of the drainage from soils which had received applications of soluble salts, concluded (45) that diffusion played an important part in the distribution of soluble fertilizers and other soluble salts in the soil. Later investigations do not, however, show that diffusion affects materially the movement of plant-food. Thus Müntz and Gaudechon say (32) that the soil may be considered as a discontinuous medium, that diffusion is extremely slow and that zones of very different composition may exist in the soil for very long periods. By placing soluble salts upon the surface of soil, adding water and studying the salt movement, they found that diffusion was downward and not lateral, except when the soil was drenched. Demolon and Brouet (8) agree with Müntz and Gaudechon and make the statement that there is apparently little danger of loss of nitrates in strong soil during the period of plant growth. Pettera's work (35) agrees with this, as does also the work of Malpeaux and Lefort (31). Sanborn obtained results in Utah from which he concluded (38) that there was no appreciable lateral movement of irrigation water. This may easily be true in most soils even though the horizontal movement of water in peat is very rapid, as reported by Franklin (14).

Work with lysimeters has been done by a number of investigators and in every case phosphorus is the plant-food element suffering least loss under all conditions and, when comparison has been made, less loss of all elements occurred when a crop was on the ground than when the land was fallow. Furthermore, von Feileitzen found (13) that there was less loss when the land was in grass than when in any other crop.

Robinson, after studying the results of many analyses, says (36) that phosphorus concentrates in the surface while magnesium and potassium concentrate in the subsoil.

Literature relating to phosphorus

There has been more work done on the study of phosphorus and more has been written on this phase of the subject than on any other. The literature of this work is voluminous and for convenience it may well be considered in accordance with the principal lines of attack.

The chemical, physical, biological, or other ways in which phosphorus is fixed, are important in that this knowledge is necessary in order to understand what must be done to increase the availability of phosphorus to growing crops. Compounds of iron, aluminum, calcium and magnesium are generally considered the ones most important in the fixation of phosphorus and it is generally believed, too, that when fixed by iron or aluminum it becomes much less available than when fixed by calcium or magnesium. Brackett and Freeman (2) found that the formation of tricalcium phosphate begins at once when ground limestone and acid phosphate are mixed. From the chemical standpoint, the phosphorus is thus made less soluble. Many trials have

been made by cultural means to determine whether lime in its several forms serves to make phosphorus more soluble or less so. Some have concluded that the tendency is for phosphorus to be made insoluble and unavailable, some believe that calcium when added to the soil, by replacing, to some extent, the iron and aluminum combined with phosphorus, makes the phosphorus more available; while still others find that the addition of lime in its various forms either has little or no effect or is beneficial in some instances and injurious in others. It is possible that not enough of the different factors are taken into account in these studies and what may appear to be the effect of calcium compound may, in reality, be due to other causes.

It has been shown that the use of fertilizer salts affects the solubility of phosphorus; those, like ammonium salts, which leave an acid in the soil tending to make phosphorus more soluble, provided, as shown by Schulov (42), the phosphate and ammonium salts are in close contact; while those which leave an alkali in the soil tend to make the phosphorus less soluble. De Jongh believes that iron and aluminum will fix phosphorus, making it insoluble and unavailable, but concludes (7) from his observations that colloids must play an important part in preventing the leaching and rapid loss from the soil of phosphates, as well as other soluble materials, at the same time holding it in such a form that it is available to plants.

Soil bacteria are also active in dissolving phosphorus and in using soluble phosphorus in their structure, thus making it unavailable to higher plants until the bacteria themselves decay.

The organic matter of soils, "humus," may fix phosphorus, as found by Dumont (9) and Karpizov (25). In this case it would again become soluble and available to plants when the organic matter itself is broken up by decay. This may be an important way in which the loss of soluble phosphorus from the surface soil is prevented. Indications that this will not always take place in all soils are furnished by the work of Petit (34).

Attempts have been made to determine the loss of phosphorus by analyzing the drainage from fertilized and unfertilized, cropped and uncropped soils. In some cases lysimeters have been used, in other cases the drainage from field plots has been analyzed. In all cases the phosphorus present in the soil, or that added, was found only in traces or not at all in the drainage, and when found at all, there was less from soil bearing a crop than from fallow soil.

In much of the investigational work, cultural methods have been used for determining the solubility of soil phosphorus. Schreiber found (40, 41) that but a small percentage of phosphorus added in a soluble form can be taken up by a crop which immediately follows, a fact which is quite commonly known. Gedroits found (15) that the order of availability of three compounds of phosphorus were: first, aluminum phosphate; second, calcium phosphate; and third, iron phosphate. Burlison found (3) that tricalcium phosphate was quite readily available to legume crops and the practice in phosphate fer-

tilizing among many farmers, especially in the middle western part of the United States, is based upon the availability of the phosphorus of tricalcium phosphate.

That soluble phosphorus is fixed in the soil must now be admitted by all, but Paturel (33) and Ullmann (44) believed that soluble phosphates remain in soluble form in the soil for long periods. This view is not generally held, and Crawley found (5) that after adding soluble phosphates to layers of soil of different depths and then leaching with water, immediately in one case and after fifteen hours in another, the amount of phosphorus held in one inch amounted to 53.35 to 99.18 per cent, while the amount held in a 6-inch layer amounted to 97.22 to 99.66 per cent. Dusserre and Bieler conducted experiments in which they found (10) that, in general, 96.6 per cent of soluble phosphorus added was retained in the first 8 cm. of soil.

In connection with this work, showing the rapid fixation of phosphorus, it is interesting to note the work of Ellett and Hill in which they find (12) that phosphorus, after being allowed to become "fixed" by calcium carbonate, magnesium carbonate, iron hydroxide or aluminum hydroxide, was yet available as a source of phosphorus to oats, wheat and corn. If this is true, it indicates that the fixation of fertilizer phosphorus in the surface soil within easy reach of plant roots is an important process and points toward the practicability of using insoluble phosphate fertilizers.

Attempts have also been made to determine the solubility of phosphorus of soils and of different phosphorus compounds in water and salt solutions, it usually being found that small amounts are soluble in pure water. Schlöessing found (39) that there was more water-soluble phosphorus in a phosphorus-rich soil than in a phosphorus-poor soil, and that, in either, there was more before than after a corn crop. He found, also, that plants were able to extract more phosphorus from either a rich or a poor soil than could be extracted by water.

Literature relating to nitrogen

It may be possible that nitrogen can, in some instances, be lost from the soil in the form of ammonia, as suggested by Lemmerman and Fresenius (29) and by Grandeau (17), but soils absorb ammonia, and nitrifying bacteria readily change it into the form of nitrates, so that by far the greatest loss of nitrogen occurs as a result of the leaching out of nitrates, which are soluble. Lysimeter and field drainage analyses show that considerable amounts may be lost in this way under certain conditions, although Demolon and Brouet (8) and Malpeaux and Lefort (31) agree that the loss of nitrogen as nitrates is overestimated and that there is no great loss of nitrates, especially during the time crops are growing on the land. As mentioned in connection with analyses of soil for phosphorus, the surface soil is richer in nitrogen than are lower depths and, since plant roots can not bring nitrogen up from below, much of it, when added to soil as fertilizer or naturally by legumes, must be retained

in the surface in one or more ways. Perhaps one of the greatest factors tending to prevent its loss is the absorption of it by plants which, in their use of it, just as with phosphorus, make it insoluble; and if other plants are ready to take it up when it is liberated from old plant residues, its loss is lessened if not entirely prevented.

Literature relating to potassium

When soluble potassium is added to the soil, it is in some way held there and does not readily wash out. Dusserre and Bieler report (10 experiments in which they find that 72 per cent of the potassium added to the soil is retained in the first 8 cm. when applied at the rate of 376 pounds of the chloride per acre and leached immediately. Crawley and Duncan report (6) that 80 per cent of the potassium applied as the sulfate was retained in the first 2 inches. Others, too, have reported experiment showing the fixation of potassium, but drainage from lysimeters and from plots always shows the presence of potassium, sometimes in comparatively large amounts. It is reported that lime in its different forms, tends to make potassium soluble, but there are many experiments which indicate that the solubility of potassium may be unaffected or even decreased by lime applications. As with nitrogen, less potassium leaches into the drainage water while a crop is growing on the land, but it is known that the potassium is not so firmly fixed in the plant tissues and is partly washed out when the plants ripen, and more completely before decay is very far advanced. Rousseaux and Brioux found (37) that, while potassium was more quickly fixed in soils than was phosphorus, it was also more easily removed by leaching. The action of bacteria, as mentioned by Koch (26), is such as to render potassium more soluble, as well as phosphorus.

Taking all of these agencies into account and noting that the analyses of soils giving total amounts of potassium, as reported from various sources, do not indicate that potassium accumulates in the surface, we might expect less of the added potassium than of the added phosphorus to remain in the surface soil. Robinson concluded (36) from the results of many analyses that potassium concentrates in the subsoil. Even though it is true that potassium leaches from the soil, there are still such large amounts in most soils that if it can be made available fast enough, it is sufficient to last into the future as long as the human race need plan.

Literature relating to magnesium

Not so much attention has been given to the question of magnesium, but Collison says (4) that it appears in fairly large amounts in the drainage water from lysimeters, and Goessmann found (16) that whenever potassium chloride was used as a fertilizer, "exceptional quantities of chloride of calcium and magnesium" were present in the drainage water. The fact that more is lost

from fallow than from cropped land, as reported by Lyon and Bizzell (30), and that arid soils contain more of it than do humid soils, shows that it is subject to loss by leaching and to conservation to some extent by crops, as are other plant-foods.

Literature relating to calcium

It is well known that calcium in the form of carbonate and other salts is washed from the soil. Hanamann estimates (20) that the loss amounts to from nearly 2700 to over 3500 pounds per acre from soils rich in lime, while the analyses reported by Hall and Miller (19) indicate a loss of 700 to 800 pounds per acre without ammonium fertilizers and nearly 1200 pounds per acre when ammonium salts were used.

Loss of limestone is caused not only by ammonium salts, but also by potassium and other salts which leave an acid in the soil. Crops do not take up large amounts of calcium in comparison with the amounts lost by leaching, legume crops taking up more than any other as reported by Lawes and Gilbert (28), by Hopkins (23, 24), and by others. It is to be expected that calcium and its compounds would not accumulate in the surface, and when leached from the surface would not accumulate in lower depths, because when once in solution the tendency would be for it to stay so on account of the carbon dioxide. It would then be carried on out of the subsoil into the drainage.

EXPERIMENTAL

By determining the total amount of the various plant-food elements in samples of soil from the surface stratum and from one or two strata below the surface of plots to which fertilizers have been applied regularly for a number of years and also from comparable check plots to which no fertilizer has been applied, it would seem that one should be able to determine whether or not the fertilizer elements added have remained in the surface to increase the amount there, penetrated to the subsurface and become fixed there, or have been entirely removed from the soil by leaching and cropping.

For the purpose of making such a study, analyses were made of soil samples collected from several sections of the "Park" plots at Rothamsted, England, by Dr. J. H. Pettit, in August, 1909, and samples collected by the author through the kindness and assistance of Professors Gardner and Worthen of Pennsylvania State College, and Director Thorne of the Ohio Agricultural Experiment Station, who also furnished the author with otherwise unavailable information regarding yields of the plots sampled, from the 4-year rotation fertility plots at State College, Pennsylvania, and from the 5-year rotation plots at Strongsville, Ohio.

The Rothamsted plots

The collection notes for the samples taken from the "Park" grass plots will give a good idea of how and where the samples from the Rothamsted experiment fields were taken, and also something as to the treatment given each plot.

It is definitely known from records that this land has been in pasture continuously during the past 300 years. It is not known that seed has ever been sown on these plots. In 1856 experiments were started to show the effect of large amounts of various plant-food materials upon the character of the herbage. These treatments are still being continued. Topographically, the plots are extremely uniform. When sampling this uniformity was noted in the soil, as well, of the plots sampled:—Nos. 3, 4–1, 7, 9 and 12. In fact, in a letter from J. H. Pettit dated Harpenden, Eng., Aug. 26, 1909, he says—"With the exception of plot 7, I found no stones or flints at all in the first two depths, while on all plots they occurred in the third depth. In fact so regular was this that often the striking of a stone was as good an indication that the 6-inch mark had been reached as was the mark on the auger." In the early days a heavy application of chalk was made upon the north end of the plots.[3] Later in the eighties applications of lime were made on the west one-half of the plots. In 1903 and 1907 lime was applied to the south one-half of plots 1 to 4–2, 7 to 11–2, 13 and 16. Accordingly when plots 3, 4–1, 7, 9 and 12 were sampled in August, 1909, it was thought best to divide plot 12 north and south and plots 3, 4–1, 7 and 9 east and west and take a separate set of samples (A and B) from each half plot.

On plots 3, 4–1, 7 and 9, four rows of five borings each were made from north to south across each half of each plot, and on plot 12, two rows of ten borings each were made on each half. The samples were dried in the Rothamsted laboratory and packed for shipment.

The difference in herbage on these plots is remarkable. At the time of sampling there were no legumes on plot 9 except a single bunch of vetch on the extreme north end. Plot 7, on the other hand, was covered with legumes. Considerable quantities of sorrel were growing upon plot 9, on both the limed and unlimed ends though the growth was much ranker on the unlimed end.

Further details of the treatment given the plots sampled as reported by Lawes and Gilbert (27) are shown in table 1.

A footnote in an article published in 1880 by Lawes and Gilbert (27) reads as follows, "'Superphosphate of lime,' always composed as under, per acre; 200 pounds bone-ash, 150 pounds of sulfuric acid (sp. gr. 1.7), and water." Hall states in "An Account of the Rothamsted Experiments" (19, p. 51), that the applications of superphosphate have been 3.5 cwt. (392 pounds) per acre. It is probable that the difference of 42 pounds between this and the amount of bone-ash and sulfuric acid reported by Lawes and Gilbert is the amount of water which they also mention. Dyer estimates (11) the total amount of phosphoric acid in 50 392-pound applications of superphosphate added to Broadbalk plot No. 7 to be 3107 pounds, which would give a composition of about 7.0 per cent of phosphorus for the superphosphate. This percentage composition for the superphosphate added to the Park plots would make a yearly application of 27.44 pounds of phosphorus per acre, or, for the 54 years the plots have been under experimentation, a total application of 1482

[3] In sampling, this area was avoided.

TABLE 1

Treatment of the divisions of the plots sampled

PLOT			REMARKS
3A	West half limed in the eighties	Lime 1903 and 1907	Unfertilized, 1856, and since
3B	West half limed in the eighties	Unlimed thereafter	
4–1A	West half limed in the eighties	Lime 1903 and 1907	Sawdust 3 years, 1856–58; superphosphate of lime, 1859 and each year since
4–1B	West half limed in the eighties	Unlimed thereafter	
7A	West half limed in the eighties	Lime 1903 and 1907	Sulfates of potassium, sodium, and magnesium and superphosphate of lime every year
7B	West half limed in the eighties	Unlimed thereafter	
9A	West half limed in the eighties	Lime 1903 and 1907	Ammonium salts in addition to the treatment given plot 7, every year
9B	West half limed in the eighties	Unlimed thereafter	
12A	Limed in the eighties	Unlimed thereafter	Unfertilized, 1856, and since
12B	Untreated		

pounds. Since sufficient analyses have not been made, or at least have not been reported, it is impossible to determine exactly the amount of phosphorus removed from each of the plots in crops. However, Hall gives (18) the average yields of the different plots for 47 years, 1856–1902 (44 years, 1859–1902, for plot 4–1).[4] Averages for the last ten years of this period and for 1902 alone also are given, and if we assume that the 1902 yield represents approximately the yields for the seven years 1903–1909 inclusive, the total yields of each of the plots sampled for the whole 64 years would be:

PLOT	TREATMENT	YIELD
		cwt.
3	Untreated	1109.1
12	Untreated	1248.1
4–1	Superphosphate alone	1158.2*
7	Mineral manures	2198.8
9	Mineral manures and ammonium salts	2931.2

* See footnote [4], page 290.

[4] Superphosphate was not applied to plot 4–1 until 1859, sawdust having been applied in 1856, 1857 and 1858. For this reason the figure given for plot 4–1 is for only 44 years.

Report is also made of the percentage composition of the crops grown on the different plots for the 18-year period 1865–1873. These percentages are given in table 2.

TABLE 2

Percentage composition of the mixed herbage of grass land (first crops), grown by different manures; including the composition of the pure ash-means of analyses of mixed-year samples

PLOT NUMBER	TOTAL FOR PERIOD OF 18 YEARS, 1856–1873				
	N	P	K	Mg	Ca
3	1.66	0.160	1.115	0.194	0.867
4–1	1.58	0.282	1.096	0.199	0.838
7	1.74	0.277	2.304	0.169	0.683
9	1.55	0.333	2.139	0.149	0.428

This table shows that the crops from 4–1, 7 and 9, plots which received phosphorus fertilizer, contained an average of 1.57 times as large a percentage of phosphorus as did those from plot 3, which received no phosphorus fertilizer. If each of the crops removed from these plots contained an average of the above percentages of phosphorus, then the total number of pounds per acre removed during the period of the experiment up to and including the year 1909 were as follows:

	lbs. per acre
Plot 3	199
Plot 4–1	366
Plot 7	682
Plot 9	1093

The composition of the crops grown on plot 12 is not reported, nor has a division of the plots been made into the limed and the unlimed parts. However, some interesting comparisons may be made.

From the estimated additions and removals of phosphorus, even though some assumptions have been made, it is clear that more phosphorus has been added to each of the treated plots during the time they have been under experimentation than has been removed, and the difference may be as much as 389 pounds per acre on plot 9 yielding heaviest and 1116 pounds per acre on plot 4–1 which yields less than half that of plot 9. Table 3 gives the total pounds per acre (900,000 pounds) of plant-food elements as found by analyses of the samples from these plots. These show that the two halves of both plots, 3 and 12, contain about the same amounts of phosphorus in each of the depths and very much less in the surface than in the surface of the other plots. It should be noted, too, that there is less in the 3 to 6-inch stratum than in either of the other two. In the soils which have been treated, there is a nearly gradual decrease in the amount of phosphorus present from the surface down.

Since the two untreated plots contain nearly the same amounts of phosphorus in comparable depths, they may be averaged, and for like reasons, the two

TABLE 3

Plant-food elements in the soils of the Rothamsted Park plots; average pounds per acre in 900,000 pounds of soil

LABORATORY SOIL NUMBER	PLOT NUMBER	TOTAL ORGANIC CARBON	TOTAL NITROGEN	TOTAL PHOSPHORUS	TOTAL POTASSIUM	TOTAL MAGNESIUM	TOTAL CALCIUM	LIMESTONE PRESENT	$CaCO_3$ EQUIVALENT TO ACIDITY PRESENT
				0 to 3 inch Stratum					
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
3,757	3B	46,422	3,384	522	9,918	2,394	3,987		81
3,760	3A	36,927	3,105	549	10,269	2,160	4,644	2,205	
3,763	4–1B	40,203	3,231	1,215	10,062	2,223	4,707		63
3,766	4–1A	39,150	3,195	1,260	10,467	2,358	6,435	2,709	
3,769	7B	33,687	2,835	927	11,889	2,395	2,196		99
3,772	7A	29,466	2,628	882	11,124	2,547	4,482	1,395	
3,775	9B	58,608	4,752	1,458	10,476	1,674	1,098		1,845
3,778	9A	53,019	4,086	1,395	9,738	2,313	1,881		1,035
3,781	12B	36,180	3,015	540	9,936	2,484	3,375		54
3,784	12A	38,448	3,087	567	9,819	2,367	3,177		63
				3 to 6 inch Stratum					
3,758	3B	27,738	2,430	486	10,260	2,286	4,392		45
3,761	3A	28,071	2,376	513	10,395	2,682	3,519	963	
3,764	4–1B	27,378	2,412	828	10,287	2,286	3,789		36
3,767	4–1A	27,981	2,448	918	10,710	2,052	4,284	1,296	
3,770	7B	23,535	2,133	828	11,727	2,277	2,124		54
3,773	7A	25,317	2,178	819	10,998	2,412	3,645		36
3,776	9B	21,681	2,097	810	11,106	2,610	2,160		1,872
3,779	9A	26,892	2,394	864	10,170	2,619	1,665		1,503
3,782	12B	26,901	2,385	477	10,089	2,700	3,582		45
3,785	12A	25,407	2,313	459	10,179	2,754	3,186		36
				6 to 9 inch Stratum					
3,759	3B	17,658	1,521	522	10,611	2,304	3,168		27
3,762	3A	19,710	1,800	540	10,773	2,079	3,042		27
3,765	4–1B	17,469	1,602	630	10,782	2,277	2,970		18
3,768	4–1A	20,709	1,782	756	10,854	2,403	3,222		18
3,771	7B	15,444	1,431	693	11,880	2,448	2,169		27
3,774	7A	19,521	1,755	657	11,007	2,574	3,159		27
3,777	9B	15,705	1,557	594	11,133	2,448	2,430		927
3,780	9A	20,925	1,863	675	10,250	2,673	2,601		990
3,783	12B	16,848	1,620	504	10,548	2,835	3,132		18
3,786	12A	16,587	1,566	531	10,647	2,385	2,394		27

halves A and B of the other plots may be averaged for the study of phosphorus. When this is done we get the figures shown in table 4.

From this table we can see how much more phosphorus the treated plots contain than the average of the untreated and, if the phosphorus added in excess of the amounts removed in crops has remained in the soil, this excess

should approximate the difference found between treated and untreated plots, provided, of course, the plots were uniform in this respect at the start. Table 5 brings these figures together.

TABLE 4

Pounds of phosphorus per acre (900,000 pounds)

PLOTS AVERAGED	DEPTH OF STRATUM	PHOSPHORUS	PLOTS AVERAGED	DEPTH OF STRATUM	PHOSPHORUS
	inches	*lbs*		*inches*	*lbs.*
3A, 3B, 12A and 12B...	0 to 3	545	7A and 7B.....	0 to 3	905
	3 to 6	484		3 to 6	824
	6 to 9	525		6 to 9	675
Total.....		1,554	Total.....		2,404
4–1A and 4–1B.....	0 to 3	1,238	9A and 9B.....	0 to 3	1,427
	3 to 6	873		3 to 6	837
	6 to 9	693		6 to 9	635
Total.....		2,804	Total.....		2,899

TABLE 5

Pounds of phosphorus per acre in treated soil over the average of that in the untreated plots 3 and 12: and calculated amount of phosphorus added to treated plots over the amount removed in crops from these plots

PLOT NUMBER	DEPTH OF STRATUM	PHOSPHORUS BY ANALYSIS	PHOSPHORUS CALCULATED
	inches	*lbs.*	*lbs.*
4–1.....	0 to 3	693	
	3 to 6	389	
	6 to 9	168	
Total.....		1,250	1,116
7.....	0 to 3	360	
	3 to 6	340	
	6 to 9	150	
Total.....		850	800
9.....	0 to 3	882	
	3 to 6	353	
	6 to 9	110	
Total.....		1,345	389

The total amounts of phosphorus found in the first 9 inches of plots 4–1 and 7 over the amounts in the untreated plots, fall very close to the estimated amount added to the treated plots in excess of the amounts removed in crops.

In plot 9, there appears to be an excess of phosphorus, even in the first 3 inches, of the amount added and not removed; and for the first 9 inches there is nearly three and one-half times as much as the calculated amount in excess of that removed in crops. The cause for this cannot be determined from the data at hand. It might be possible that this plot differed from the others in this respect at the start, but that is not probable. This is the only plot receiving ammonium salts and another possible cause which suggests itself is that by increasing the vigor of the crops they have caused the plants to send roots deeper and to absorb more phosphorus from the subsoil, leaving more of the applied phosphorus in the surface. If there has been any loss whatever from any of the plots, this loss has probably been entirely prevented from this plot. Table 3 shows that while plot 9 contains more phosphorus in the first 3 inches than either plots 4–1 or 7, it contains in the second 3 inches, as an average, less than plot 4–1 and but little more than plot 7, while in the subsoil it contains less than either of the others. This may indicate that perhaps plot 9 may contain still less phosphorus than plots 4–1 and 7 in the still lower strata, which could account for the excess in the surface 3 inches.

Comparisons of the amounts of phosphorus in the surface 3 inches show that plot 7 contains very much less than either of the other two treated plots, but the larger amounts removed in crops from plot 7 than from plot 4–1 would account for the difference between these plots. The amounts of phosphorus in the second stratum of the differently-treated plots are fairly close together and so are they for the third stratum. The fact that there is more phosphorus in the third stratum of any of the treated plots than in any stratum of the untreated plots, while, at the same time, larger crops were grown on the treated plots, shows that phosphorus has been conserved even in the third stratum of the treated plots. However, at the same rate of decrease of phosphorus content with depth as that shown in the three strata analyzed, one would not have to go much deeper to find soil of approximately the same phosphorus content as the subsoil of the untreated plots. By far the largest part of the phosphorus added to these plots, in excess of that removed in crops, has remained in the surface 3 inches, and it would seem that all of it has remained in the surface foot.

The low phosphorus content of the surface 3 inches of plot 7 suggests that the addition of alkali salts to that plot has encouraged the utilization of phosphorus by shallow-feeding plants. It is an interesting fact that, as an average, the herbage on plot 7 contains 23.8 per cent of legumes and 62 per cent of grasses, while that on plot 9 contains 0.4 per cent of legumes and 88.7 per cent of grasses. [In 1902 these percentages were 55.5 per cent of legumes and 20.3 per cent of grasses on plot 7, and 1.3 per cent of legumes and 91.2 per cent of grasses on plot 9 (18)].

Comparison shows a fairly close correlation between the content of organic carbon and of nitrogen on all plots. The surface of plot 9 is high in these two elements and plot 7 is low, showing also something of a correlation with

the phosphorus content. Plot 3B shows a somewhat abnormally large amount of both organic carbon and nitrogen in the surface, which is not easily accounted for.

In both the second and third 3-inch strata, the variations found in the surface are eliminated and there is a fair degree of uniformity over all of the plots, both treated and untreated.

The potassium content of all those plots receiving no potassium fertilizer, increases fairly gradually with depth through the three strata (table 3). The potassium added to the other plots has been in the form of potassium sulfate and was applied at the rate of 300 pounds per acre for the first 23 years, 1856–1878, and 500 pounds per acre for the last 31 years, 1879–1909, making a total of 22,400 pounds for the whole period. Commercial potassium sulfate usually contains about 40 per cent of potassium and, at this percentage, the potassium added during the 54 years has amounted to 8960 pounds per acre, where used. If the amounts of potassium in each of the strata of the different

TABLE 6

Increase of potassium on treated soil plots due to treatment as found by soil analyses

DEPTH OF STRATUM	PLOT			
	7B	7A	9B	9A
inches	*lbs.*	*lbs.*	*lbs.*	*lbs.*
0 to 3	1,903	1,138	490	−248
3 to 6	1,496	767	875	− 61
6 to 9	1,235	362	488	−394
Total.....	4,634	2,267	1,853	−703

untreated plots 3B, 3A, 12B and 12A are averaged, the following pounds per acre are found:

0 to 3-inch	9,986
3 to 6-inch	10,231
6 to 9-inch	10,645

If, now, we subtract from the potassium content of each of the different strata of the treated plots the potassium content of the corresponding stratum of the untreated plots, we will get an estimated amount of potassium increase due to treatment. These amounts are given in table 6.

If that part of the added potassium which has not been removed in crops has remained in the surface 9 inches of soil, then the total amounts given in the above table should agree with the amounts added in fertilizer in excess of that removed in crops. Unfortunately for this comparison, we do not have the yields of each half of the plots separate, but for the whole of plot 7, the calculated amount added in excess of that removed has been 3286 pounds per acre, while that for plot 9 has been 1938 pounds. The amount for plot 7, 3286

pounds, approximates fairly closely the average gain in potassium of the two halves of plot 7, 3450 pounds, but it will be noticed that the actual increase of potassium in plot 7 B, without lime, is 1348 pounds above the calculated amount, while the actual increase for plot 7A, with lime, is 1019 pounds less than the calculated amount. The agreement between the actual and the calculated increase for plot 9B, receiving ammonium salts without lime, is close, but the actual increase is slightly lower, and plot 9A receiving both ammonium salts and lime, contains 803 pounds of potassium less than do the check plots while, calculating from the applications and the crops removed, there should have been an increase of approximately 1938 pounds.

In plot 9B, receiving ammonium salts and in plot 9A receiving ammonium salts and lime, there is an increase in potassium content in going from the first to the second stratum, much the same as in the untreated plots, while in plot 7B, and in plot 7A to a less extent, there is more potassium in the first than in the second stratum. Those plots which have received potassium fertilizers and show an increase in potassium content, show an increase in the second and third strata, while the plot showing a decrease, shows a greater decrease in the third stratum than in either the first or the second.

The conclusion that must be drawn is that though potassium is fixed in the surface, its loss is easily brought about by methods of treatment. Table 3 shows, however, that if there are means of making the potassium available, there is room for many times the depression suffered by any plot before it approaches exhaustion.

The magnesium content of the different plots bears no apparent relation to the applications made. If the magnesium sulfate added contains 10 per cent of magnesium, the total application of magnesium has amounted to only 540 pounds for the 54 years. The crops removed from the plots receiving magnesium contain a lower percentage of this element than do the check plots, though the absolute amounts removed from the treated plots have been about twice that removed from the check plots. Perhaps the most noteworthy point is that where ammonium salts have been used, the magnesium in the surface 3 inches has been decreased, even in spite of the application of magnesium fertilizers. This is particularly true of plot 9B. Other than this, there seems to be but little that can be said, since the amount of magnesium added is small and the variation in content of magnesium is greater within those plots receiving none of it than between those receiving it and those receiving none, excepting plot 9B.

It has already been quite definitely shown that the tendency for calcium is toward a gradual movement downward and out of the soil. Table 3 shows that the calcium content corresponds very well to the content of carbonate or the acidity developed. Plot 4–1B seems to be an exception to this, but even in this plot the amount of calcium is not high and the acidity developed is not great. Although, as stated in the collection notes, lime was applied to the west half of the plots in the early eighties, the calcium and acidity

content of plot 12A and 12B indicate that the east and the west halves have again become uniform in this respect. The effect of the lime applied in 1902 and 1907 is still visible on all plots. There is not more calcium in the soil of plot 3A than in that of plot 4–1B which has not received lime, but plot 3A contains carbonates in each of the first two strata, while plot 4–1B has a greater lime requirement in the surface than in either of the other two strata.

Plots 3A, 4–1A and 7A show that so long as applied limestone remains in the soil at all, to the depths sampled and very probably to much greater depths, by far the greatest amount of it is in the surface. That it does penetrate to the second stratum is shown, however, by plots 3A and 4–1A. The effect of ammonium salts in causing a loss of both calcium and carbonates is well illustrated on plots 9B and 9A. Either of these plots has a much greater lime requirement in all three strata than has any of the other plots and the calcium content in the surface of either is lower than that of any other plot, even though lime has been applied to plot 9A. This lime has, however, made the lime requirement of plot 9A considerably less than that of plot 9B. It appears from plot 7B that the mineral salts used have caused a loss of calcium in all three strata without having very materially increased their acidity.

In so far as the results of tests made on these Park plots show, the movement of the different elements, even downward, is very slow. The practice which is followed in adding fertilizers indicates that there is no appreciable lateral movement. Adjoining plots are not separated by division strips, but when fertilizers are applied, a canvas sheet is put up along the division line to prevent the blowing of material upon plots where it should not be and, as shown by the plot yields, one plot receives no benefit from the fertilizers added to any of the adjoining plots.

The Pennsylvania plots

The first of the experimental crops were removed from these plots in 1882, so that 1915 completed the 34 years for which we have records of the yields and treatment given to each plot. A diagram of the plots and the treatment which each has received are given in the annual report of the Pennsylvania State College for 1911–1912, p. 84–86. The plots chosen for study were plots 1 and 14, both check plots receiving no treatment, plot 3 receiving only phosphorus, and plots 9 and 17 receiving full treatment of nitrogen, phosphorus and potassium. Each of these plots on each of the four series (I, II, III, IV) were sampled in three depths, 0–$6\frac{2}{3}$ inches, $6\frac{2}{3}$–$13\frac{1}{3}$ inches and $13\frac{1}{3}$–20 inches, about eighteen borings to each plot, each depth being kept separate and all analyses made on each sample separately.

The samples taken from the treated plots appeared to be very much like the check plots with which they are compared. Because of some non-uniformity in plot 14, series 4, part of the plot was left unsampled. By doing so, a better check sample with which to compare samples from plots 9 and 17 was obtained.

The yields of these plots were obtained from the annual reports of the Pennsylvania State College for the years 1901–1902, 1907–1908, 1911–1912 and from yields furnished by Professor Worthen. Table 7 gives the total number of applications of fertilizers made, the rate and the total amounts of the three elements, nitrogen, phosphorus and potassium. Table 8 gives the total

TABLE 7

Fertility treatment given the Pennsylvania plots, Series I, II, III, IV

PLOT NUMBER	NUMBER OF APPLICATIONS	ELEMENTS APPLIED	RATE, POUNDS PER ACRE	TOTAL POUNDS PER ACRE
1				
3	17	P	20.96	356
9	17	N	24.00	408
9	17	P	20.96	356
9	17	K	83.00	1,411
14				
17	17	N	24.00	408
17	17	P	20.96	356
17	17	K	83.00	1,411

TABLE 8

Pounds per acre of phosphorus removed from each plot in crops

SERIES NUMBER	PLOT 1	PLOT 3	PLOT 9	PLOT 14	PLOT 17
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
I	131.6	203.8	259.0	176.6	276.6
II	159.4	226.7	312.6	226.1	260.5
III	147.2	228.5	284.9	166.6	250.1
IV	198.3	234.5	302.5	166.3	257.5

TABLE 9

Net gain (+) or loss (−) of phosphorus per plot, 1882–1915, as a result of fertilizing and cropping

SERIES NUMBER	PLOT 1	PLOT 3	PLOT 9	PLOT 14	PLOT 17
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
I	−132	+152	+97	−177	+ 79
II	−159	+129	+43	−226	+ 95
III	−147	+127	+71	−167	+106
IV	−198	+121	+53	−166	+ 98

amounts of these three elements removed from each of the plots in crops, taking as the average amounts of the three elements in the different crops, the amounts given by C. G. Hopkins, in "Soil Fertility and Permanent Agriculture," p. 154. Table 9 gives the net gain or loss of phosphorus as a result of fertilizing and cropping.

If, at the beginning of the field experiments, each of these Pennsylvania plots had contained the same amount of phosphorus in each of three strata sampled, and if the crops removed from all plots had contained the same percentage of phosphorus, then it would be an easy matter to determine the proportion of added phosphorus remaining in each of the layers and to trace its movement downward. Thus a comparison of plots 1 and 3 in series III shows that plot 1 has had 147 pounds of phosphorus removed from it in crops with no additions made, while plot 3 has had 127 pounds more phosphorus added in fertilizers than removed in crops, thus making a difference of 274 pounds which plot 3 should contain more than plot 1. Analyses of these two plots show that plot 3 contains, in the first stratum ($6\frac{2}{3}$ inches), 240 pounds more phosphorus than plot 1; in the second stratum 20 pounds, and in the third stratum 20 pounds, a total of 380 pounds which very closely approximates the difference of 274 pounds found by the above estimation. In this instance it would be indicated that on plot 3 about 86 per cent of the added phosphorus plus that available in the soil, as indicated by the amount removed from check plot 1, has been retained in the first $6\frac{2}{3}$ inches or the plow depth, while the next two strata of like extent contain only about 7 per cent each.

It is not safe to rely too much on this assumption, however, and, unfortunately, we do not have the phosphorus content of these three strata at the beginning of the field experiments nor the composition of the crops removed from the different plots. In general, however, the phosphorus content of the different plots must have been similar, though there may have been some variation, and for the present we can not do better than to use an average composition for all crops.

While this comparison can not be accurately made, table 11 is made up in such a way as to show the difference between check and treated plots as calculated from the amounts removed in crops and the amounts added in fertilizers, also the amounts found by analyses in treated plots more than in check plots (+) or less than in check plots (−) in each of the sampled strata. The total amounts of phosphorus found in the different strata are given in table 10.

Although there is but one comparison in which the two figures agree so well as in the above-mentioned case, we are able to obtain some quite definite and valuable information which points qualitatively, if not quantitatively, to the same conclusion as that reached in considerinng plots 1 and 3, series III.

Plots 1 and 3 being located nearer each other than any other check and treated plots, these were first considered on all the series, and for the same reason plots 14 and 17 were next taken. Plot 9 was compared with the average of check plots 1 and 14.

Comparison shows that in only one case does a check plot contain more phosphorus in the surface stratum than does a treated plot with which it is compared, on series I, plots 9 and 14. In all other cases the treated plot contains from 100 to 400 pounds, or from 11.1 to 54.3 per cent, more phos-

phorus than the untreated plot compared with it. In the subsurface, the phosphorus content of the check plot is, in four of the twelve comparisons, higher than the treated plots with which they are compared, while the dif-

TABLE 10

Pounds of phosphorus pe acre (2,000,000 pounds) in the soils from Pennsylvania, by analysis

PLOT NUMBER	0 TO 6⅔ INCH STRATUM	6⅔ TO 13⅓ INCH STRATUM	13⅓ TO 20 INCH STRATUM	TOTAL
	Series I			
	lbs.	*lbs.*	*lbs.*	*lbs.*
1	900	660	580	2,140
3	1,140	760	600	2,500
9	960	700	620	2,280
14	1,080	880	680	2,640
17	1,400	1,100	800	3,300
	Series II			
1	900	660	660	2,220
3	1,000	720	640	2,360
9	1,140	820	700	2,660
14	920	880	780	2,580
17	1,320	800	760	2,880
	Series III			
1	860	680	660	2,200
3	1,100	700	680	2,480
9	1,140	820	600	2,460
14	780	620	620	2,020
17	1,040	720	620	2,380
	Series IV			
1	900	760	660	2,320
3	1,100	720	640	2,460
9	1,080	680	580	2,340
14	700	620	560	1,880
17	1,000	740	640	2,380
	Average for all series			
1	890	690	640	2,220
3	1,085	725	640	2,450
9	1,080	755	625	2,435
14	870	750	660	2,280
17	1,090	840	705	2,735

ference found in the other eight comparisons vary only from 20 to 170 pounds, or from 2.9 to 26.1 per cent. In the subsoil, seven of the twelve comparisons show that the check plots contain more phosphorus than do the treated plots

with which they are compared, while the other five comparisons show the narrow variation of from 0 to 120 pounds, or 0 to 17.6 per cent.

Averaging the percentage differences between treated and check plots for all four series, comparison of plots 1 and 3 shows a difference in phosphorus content in favor of the treated plots amounting to 22.0 per cent in the first

TABLE 11

Phosphorus per acre in treated plots more than in untreated plots by analysis and calculated increase per acre of phosphorus in treated plots over untreated plots

PLOTS COMPARED		INCREASE OF 3 OVER 1		INCREASE OF 17 OVER 14		INCREASE OF 9 OVER AVERAGE OF 1 AND 4		AVERAGE INCREASE FOR THE THREE COMPARISONS	
Series	Stratum	By analysis	Calculated	By analysis	Calculated	By analysis	Calculated	By analysis	Calculated
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
I	1	240		320		−30		177	
	2	100		120		−70		50	
	3	20		120		−10		43	
	Total.....	360	284	560	256	−110	251	273	264
II	1	100		400		230		243	
	2	60		−80		50		10	
	3	−20		−20		−20		−20	
	Total.....	140	288	300	321	260	191	233	267
III	1	240		260		320		273	
	2	20		100		170		97	
	3	20		0		−40		−7	
	Total.....	280	274	360	273	450	229	363	259
IV	1	200		300		280		260	
	2	−40		120		−10		23	
	3	−20		80		−30		10	
	Total.....	140	319	500	264	240	235	293	273
Average	1	195		320		200		238	
	2	35		65		35		45	
	3	0		45		−25		7	
	Total.....	230	291	430	279	210	227	290	260

$6\frac{2}{3}$ inches, of 5.7 per cent in the second $6\frac{2}{3}$ inches, and of only 0.1 per cent in the third $6\frac{2}{3}$ inches. Corresponding averages for comparisons of plots 14 and 17 are 35.4, 12.9 and 7.3 per cent; and for the 1 and 14 average and 9, 22.7, 4.9 and −3.8 per cent. Thus it is seen that the addition of phosphorus fertilizer has, after 34 years of fertilizing and cropping, left the surface of the treated plots 26.7 per cent richer in phosphorus, as an average of all above

comparisons, than the untreated plots with which they are compared, the average difference in the subsurface being but 7.8 per cent while the average of the subsoils of the treated and the untreated plots show practically the same phosphorus content, being only 1.2 per cent in favor of the treated plots.

From these data it appears that a large part, perhaps three-fourths, of the added phosphorus has remained in the plowed soil or has been removed in crops, that nearly, if not quite, all of the remainder has not been carried lower than 13⅓ inches. Results would be still more in favor of this conclusion if, as shown by analyses of crops from the Park plots, the crops from treated land contains more phosphorus than those from the check plots, and if this had been taken into account in the above calculations.

The Ohio plots

Samples taken from the Strongsville plots were made up of composites from about ten borings and represent the same strata as those from Pennsylvania. Samples were taken from corresponding plots on series A, B, C and D, the plots being 1, 4, 10, 13, 16 and 19, all check plots receiving no treatment; plot 2 receiving phosphorus at the rate of 20 pounds per acre in 5 years; plot 11 receiving phosphorus at the rate of 20 pounds, nitrogen at the rate of 76 pounds and potassium at the rate of 108 pounds in 5 years; and plot 17 receiving these three elements at the rate of 30, 38 and 108 pounds, respectively, during the same period. Table 12 gives the total amount of these elements added from the time the experiment began to the time the samples were collected. Table 13 gives the total amount of phosphorus in each stratum sampled, as found by analyses, and table 14 shows the estimated removal of phosphorus in crops if we consider all crops to have the amount used by Hopkins (23, 24) as the average composition.

It will be seen by comparing tables 12 and 14 that in no case has as much phosphorus been added in fertilizers as has, according to this estimate, been removed in crops. If the crops from treated plots contained a larger percentage of phosphorus, the difference is still greater.

Even though more phosphorus has been removed than returned to the treated plots, that added should conserve the phosphorus originally in the soil so as finally to make the treated plots higher in this element than the check plots, unless the amount removed in crops from the treated plots is enough to equal that added in fertilizers plus that removed from the check plots. Table 15 shows the gain or loss in phosphorus in pounds per acre for treated plots over the weighted average of the check plots on each side of it, as found by analysis of the soil samples. It gives, also, the calculated amount of phosphorus the treated plots should contain more than the weighted averages of check plots based on the above calculation.

This table shows that the calculated increase of the various treated plots over the untreated ones is very small, amounting in many instances to less than the limit of analytical error, which is 40 pounds. If the treated plots produced crops containing a higher percentage of phosphorus than the average here used, and the check plots produced crops with a lower percentage composition than the average used, it might easily be that an actual loss of phosphorus would be shown for the treated plots in comparing them with the check plots, as is shown by the analytical results in several instances. With such small differences and the variations found in untreated plots, which is greater in many cases than the variation between comparable treated and

TABLE 12

Amounts per acre of fertilizer elements added to the Strongsville plots

	PLOT	P	K	N
		lbs.	*lbs.*	*lbs.*
Series A	2	80		
	11	80	448.56	304
	17	110	448.56	117⅓
Series B	2	80		
	11	80	432.00	304
	17	115	432.00	164⅔
Series C	2	80		
	11	80	390.46	278⅔
	17	110	390.46	139⅓
Series D	2	80		
	11	80	456.92	299⅓
	17	120	456.92	201⅔

untreated plots, nothing better can be done than to compare the amounts in the different strata as shown in table 13. In general, much more phosphorus is found in the surface than in either the second or the third stratum. In some cases the next largest amount is in the second, and in other cases, in the third stratum. The sample from the second stratum of plot 17, series B, and from the third stratum of plot 10, series C, are abnormally high, possibly because of the inclusion in these samples of phosphatic material that is there as a contamination from some unknown source. Otherwise, there is less fluctuation in the content of phosphorus in the two lower strata of the different plots than there is in the surface stratum.

TABLE 13

Pounds per acre (2,000,000 pounds) of phosphorus in the soil samples from the Ohio plots

PLOT NUMBER	0 TO 6⅔ INCH STRATUM	6⅔ TO 13⅓ INCH STRATUM	13⅓ TO 20 INCH STRATUM	TOTAL
		Series A		
	lbs.	*lbs.*	*lbs.*	*lbs.*
1	820	340	480	1,630
2	920	420	440	1,780
4	740	400	420	1,560
10	1,020	280	280	1,580
11	1,060	440	360	1,860
13	740	640	340	1,720
16	740	380	360	1,480
17	1,000	440	360	1,800
19	1,020	480	380	1,880
		Series B		
1	920	400	520	1,840
2	820	440	480	1,740
4	720	340	600	1,660
10	1,000	520	480	2,000
11	1,100	400	320	1,820
13	1,000	500	360	1,860
16	740	440	340	1,520
17	900	880	320	2,100
19	1,020	520	420	1,960
		Series C		
1	860	340	360	1,560
2	820	400	340	1,560
4	860	440	340	1,640
10	920	460	1,340	2,720
11	1,040	580	400	2,020
13	1,160	640	420	2,220
16	820	320	320	1,460
17	920	400	380	1,700
19	900	700	340	1.940
		Series D		
1	1,160	740	420	2,320
2	1,000	400	380	1,780
4	1,160	620	480	2,260
10	940	360	280	1,580
11	760	320	300	1,380
13	900	320	460	1,680
16	960	360	380	1,700
17	900	380	380	1,660
19	960	480	340	1,780

TABLE 14

Calculated amount of phosphorus per acre removed in crops from the Ohio plots

PLOT	SERIES			
	A	B	C	D
	lbs.	*lbs.*	*lbs.*	*lbs.*
1	67.7	64.5	62.2	82.6
2	117.0	86.3	94.9	128.5
4	61.6	42.7	57.0	84.6
10	77.9	83.1	70.9	85.3
11	140.9	119.3	101.9	128.7
13	75.9	71.7	69.2	94.4
16	85.5	72.0	76.4	94.7
17	139.7	116.5	114.6	139.5
19	91.2	81.7	56.7	90.6

TABLE 15

Pounds per acre gain (+) or loss (−) in phosphorus of treated over weighted average of untreated plots

PLOT NUMBER	UM	SERIES								AVERAGE	
		A		B		C		D			
		By analysis	Calculated	By analysis	Calculated	By analysis	Calculated	By analysis	Calculated.	By analysis	Calculated
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
2	1	+133		− 33		−40		−160		− 25	
	2	+ 60		+ 60		+27		−300		− 38	
	3	− 20		− 67		−13		− 60		− 40	
	Total..	+173	+28.7	− 40	+50.9	−26	+45.6	−520	+ 34.8	−103	+40.0
11	1	+133		+100		+40		−167		+ 27	
	2	+ 40		−113		+60		− 27		+ 10	
	3	+ 60		−120				− 40			
	Total..	+233	+16.3	−133	+40.0		+48.1	−234	+ 39.6		+36.0
17	1	+167		+ 67		+73		− 60		+ 62	
	2	+ 27		+413		−47		− 20		+ 93	
	3	− 7		− 47		+53		+ 13		+ 3	
	Total..	+187	+57.7	+433	+73.7	+79	+65.2	− 67	+104.0	+158	+75.1
Average	1	+141		+ 45		+24		−129		+ 21	
	2	+ 42		+120		+13		−116		+ 15	
	3	+ 11		+ 78				− 29			
	Total..	+194	+34.2	+243	+54.9		+53.0	−274	+ 55.9		+50.4

CONCLUSIONS

The conclusion must be drawn from the work presented here that when phosphorus is used as a fertilizer, it remains almost where it is placed in the soil until removed in crops or removed by some such process as erosion by water or wind action.

The addition of alkali salts (sulfates of potash, soda and magnesia) seems to encourage the utilization of phosphorus from the surface stratum, especially by legume plants, which probably also secure nitrogen chiefly from the soil air in the surface stratum.

There may be some loss of nitrogen through drainage, but when other fertility conditions are right and crops are kept on the ground all through the growing season, this loss is very small and there is a tendency for nitrogen, added in the form of ammonia, to accumulate in the surface soil, probably in plant roots and residues.

Potassium, though easily and quickly fixed in soil, is more subject to movement within the soil as a result of fertilizing with other salts, and in this way may be leached beyond the reach of plant roots.

Carbonates are rather easily washed from the soil even when no other treatment is given, but much more readily washed out when ammonium salts are used.

The loss of magnesium is brought about by the use of ammonium salts as fertilizers.

Calcium decreases with the loss of carbonates. Also, when alkali fertilizer salts are applied, it decreases more rapidly than acidity develops. Ammonium salts cause as marked a loss of calcium as of carbonates, and its loss occurs relatively as rapidly as the acidity develops.

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THE OCCURRENCE OF ACTINOMYCETES IN THE SOIL

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Received for publication October 15, 1918

INTRODUCTORY

Actinomycetes include both parasitic and saprophytic forms and can be isolated from air, water, sewage, salt lakes, milk and certain wounds, but chiefly from the soil. They form a large and important group of soil microorganisms; this is manifested by the total numbers of these organisms found in different soils, numerous species and manifold activities. Attention has been repeatedly called to the fact that in soil containing an abundance of undecomposed organic matter, such as the stubble and roots of plants and animal manures, the actinomycetes are present in large numbers greatly exceeding those in the corresponding soil poor in undecomposed organic substances. As is pointed out elsewhere (24), these organisms have such a variety of activities that they cannot be spoken of as a group, as one cannot speak of the bacterial activities as of one group, but rather of a number of species, whose functions are variable.

HISTORICAL

Globig (6) described in 1888 a thermophilic Actinomyces, isolated from garden soil upon potato as a medium, having an optimum temperature of 58°C. Rossi-Dori (20) isolated an Actinomyces from the soil that he called *Streptothrix alba*, a term commonly applied to many soil actinomycetes.

Rullman (21) studied one form in 1895 which he called *Actinomyces odorifer*, due to the production of the peculiar odor so characteristic of this whole group.

Beijerinck (1) found actinomycetes one meter deep in garden soil, two meters deep in sandy soil and in the mud of the Meuse under the river bed. He stated that these organisms are omnivorous and can live and reproduce under circumstances favorable and even unfavorable for nutrition; he also pointed out their ability to decompose organic substances, since they produce quinone, which is a carrier of oxygen and further oxidation.

Nadson (18) isolated in 1900 three Actinomyces from the curative mud of a salt lake in Russia, and studied them in detail, but unfortunately his paper seems to have been overlooked by subsequent investigators. He stated that they decomposed proteins readily with the production of ammonia and hy-

drogen sulfide; they are also characterized by the precipitation of $CaCO_3$ in large quantities.

Hiltner and Störmer (13) found in 1903 that in the fall there is an increase in the numbers of actinomycetes relative to the other groups of microorganisms due to the increase of the content of undecomposed organic matter in the soil. They form in the spring 20 per cent, in the fall 30 per cent, and dropping in the winter to 13 per cent of the total soil microbial flora; the addition of stable manure, due to its straw content, results in an increase in the numbers of actinomycetes.

Petri (19) isolated an Actinomyces from the roots of strawberries and expressed the opinion that the actinomycetes are energetic destroyers of organic matter; he has shown that they can be inoculated into plants but that they act as saprophytes, since after six months the plants were still in a healthy condition.

Gilbert (5) in 1904 isolated an Actinomyces from the soil, with potato as a medium, which grew at an optimum temperature of 50–55°C. Hiltner (12) found the actinomycetes to occur in large quantities in stable manure and ascribed to them the importance of fixing in their bodies the easily assimilable nitrogen compounds. Heinze (10, 11) speaks of the actinomycetes as playing an important part in soil fermentation. Macé (16) isolated an Actinomyces from the soil which was found to be one of the most active agents in the transformation of albuminous matter. Long before that (15) Macé reported about an Actinomyces found in abundance in water which is able to deposit calcareous secretions around its filaments.

Fisher (7) found in sandy, sandy loam and loamy soils not more than 15 per cent of the microorganisms to be actinomycetes.

Fousek (8) found that loam soils contain a higher number of actinomycetes than other soils, sandy soils coming last. He confirmed the observation of Hiltner and Störmer (13) that there is a proportionate increase of actinomycetes in fall (27 to 35 per cent) than in the spring (18 to 23 per cent), but there is no reduction in the winter. Cultivation of the soil effects a decrease in the numbers of actinomycetes; the numbers are higher in the fall because new quantities of undecomposed organic substances are added to the soil. Actinomycetes were found in forest soils and on the roots of different plants, particularly grass and legume roots, the upper cell layers of which died down. He therefore concluded that the actinomycetes are important in the decomposition of plant residues in the soil. When cultures of actinomycetes were added to the soil, a better growth of important agricultural plants was obtained because the organic substances of the soil are decomposed energetically by these organisms, thus making available larger quantities of nutrients, particularly nitrogenous compounds, for the plants. He suggested that the actinomycetes stimulate nodule formation on the roots of leguminous plants, attacking these roots, resulting in a more ready access for the legume bacteria. Hagem (9) isolated four actinomycetes and Münter (17) isolated seven species

from the soil. Conn (2) stated in 1913 that the actinomycetes may make up as many as 40 per cent of the soil bacteria. While Hiltner and Störmer (13) found as many as 2.5 millions of actinomycetes per gram of soil and Conn (2) 12 to 14 millions, Krainsky (14) obtained only 20,800 per gram, but these formed 30 per cent of the total number of microorganisms developing on the special medium used. Krainsky concluded that the actinomycetes play an important part in the decomposition and humification of plant remains in the soil. The senior writer (22) found that the actinomycetes are present in large quantities in all cultivated and uncultivated soils. Their numbers decrease with depth of soil but increase in proportion to the other soil microorganisms.

Conn (3) observed a greater number of actinomycetes in old sod soil (39.4 per cent of the microbial soil flora) than in cultivated soil (21.3 per cent). He suggested the idea that these large numbers in sod soil are due to the fact that the actinomycetes are concerned in the decomposition of grass roots. Conn (3) brought further evidence that an addition of grass roots to soil resulted in an increase in numbers of actinomycetes. This only confirms the observations previously made on the same point by Hiltner and Störmer (13), and Fousek (8).

The writers (25) found an average of 743,000 actinomycetes at a depth of 1 inch in New Jersey soils and 933,000 at a depth of 4 inches, which formed 9.2 and 15.0 per cent, respectively, of the total microbial soil flora with the culture media used. The numbers of actinomycetes regularly decrease with depth, but increase in proportion to the other microorganisms, so that at a depth of 30 inches they formed an average of 240,000 per gram, or 65.6 per cent of the total microbial flora developing on the given media. Similar results were obtained with an Oregon soil, while several California soils studied gave 380,000 to 1,890,000 of actinomycetes per gram, or 19.2 to 45.0 per cent of the total microbial population found with the given medium.

Conn (4) criticizes the work of the writers and thereby allows several unpardonable mistakes to creep in. The medium most extensively used in our work was Czapek's solution agar, which is well known to mycologists, and the description of its composition in the paper was thought to be superfluous, particularly since it has been recently given by a number of investigators in their different publications.[1] This very formula was given in a personal letter to Dr. Conn. The composition of this medium is given on page 313. But Dr. Conn ascribed to us the addition of 0.2 per cent KNO_3 to the Czapek's

[1] Dox. A. W. 1910 The intracellular enzymes of *Penicillium* and *Aspergillus*, with special reference to those of *Penicillium Camemberti*. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 120, p. 37.

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solution and the substitution of dibasic for monobasic potassium phosphate; and also, since Thom referred to a certain modification of Czapek's solution made by Dox as Dox's solution, Dr. Conn labels our Czapek's solution agar as Dox's agar. This is entirely incorrect, since the medium used by us has been referred to also by many others as Czapek's solution agar.

Dr. Conn further criticizes the fact that we laid a great deal of emphasis upon the liquefaction and pigment production of gelatin. It has been recognized by all the recent workers with actinomycetes that not much emphasis should be laid upon the growth of these organisms on organic media, but that rather synthetic media should be used, which are standard in composition and produce a more characteristic growth. But, by using synthetic media alone, necessarily one has to lay the greatest stress upon the cultural characters and the morphology of the organism. Many difficulties are encountered when one comes to study the morphology of the actinomycetes, which is a task not much simpler than that of the bacteria. It has not even been established as yet that such important characters as club or spiral formation, and size and shape of spores, are constant within a given species, and do not change with the medium upon which the organisms are grown, age of culture, and source of isolation. It is, therefore, our opinion that just as much emphasis should be laid upon the biochemical activities of the organisms as upon their morphology, until the constancy of these characters has been definitely established. Fully recognizing the importance of this fact, in our attempt to classify the different species of actinomycetes, a number of biochemical investigations were conducted, using different proteins and carbohydrates. But these investigations, to be thorough, required a great deal of time, and, since the paper was looked upon by the writers as only preliminary to a series of investigations on the actinomycetes of the soil, only the liquefaction of pure gelatin in distilled water, which is a fairly stable compound, and the pigment production on the gelatin, which seems to be also characteristic of the species, were reported.

EXPERIMENTAL

Soils used

A number of soils collected from different parts of this country have been used for this work. Complete descriptions of the soils, as well as of the fertilization and cropping were published elsewhere (23). These soils will be termed here after the locality from which they were taken. In all, 25 soils were used for the determination of numbers of actinomycetes and bacteria and for the isolation of actinomycetes. These soils are as follows: (1) New Jersey Sassafras garden soil, cultivated; (2) New Jersey Sassafras orchard soil, cultivated; (3) New Jersey clay meadow soil, uncultivated; (4) New Jersey Sassafrass forest soil, uncultivated; (5) Iowa Carrington loam, cultivated; (6) Jamesburg cranberry soil from New Jersey; (7) Louisiana sandy loam, culti-

vated; (8) California fertilized orange soil, cultivated; (9) California unfertilized orange soil, cultivated; (10) California upland soil; (11) California adobe soil; (12) California sandy loam; (13) Oregon adobe-like soil, cultivated; (14) Oregon white land, cultivated; (15) Porto Rico clay loam, cultivated; (16) North Dakota wheat soil, cultivated; (17) North Dakota flax soil, cultivated; (18) Hawaiian pineapple soil, cultivated; (19) Alaska soil; (20) Texas Lufkin fine sandy loam, cultivated; (21) Colorado alfalfa soil; (22) Maine Aroostook potato soil, cultivated; (23) Maine Aroostook soil infected with *Spongospora*; (24) Alberta, Canada, soil, cleared in 1909, under grass; (25) Alberta soil, cleared in 1911, cultivated. Soils 1, 2, 3, 10, 11, 12 and 13 are the same as used for the work published before (25).

The samples of all these soils were taken under sterile conditions and shipped in sterile containers. All the samples were plated out as soon as received at this laboratory; this took place during the period between March 1 and May 30, 1916. All soils came in a perfect condition, except the two Dakota and the Colorado soils, which dried out in shipment.

Media used

No special media are required for the bringing out of the numbers of actinomycetes in the soil; they usually develop well on the media used for isolation of soil bacteria. But for the study of the specific characters of the organisms and for the differentiation of the types closely related, special media are necessary.

Two different media have been used for this work:

1. Czapek's solution agar prepared according to the following formula:

Distilled water	1,000 cc.
Magnesium sulfate	0.5 gm.
Dibasic potassium phosphate	1.0 gm.
Potassium chloride	0.5 gm.
Ferrous sulfate	0.01 gm.
Sodium nitrate	2.00 gm.
Saccharose	30.00 gm.
Agar	15.00 gm.

2. Modified albumen agar. This medium, described in detail elsewhere (23) is made up as follows:

Distilled water	1,000 cc.
Dextrose	10.00 gm.
K_2HPO_4	0.50 gm
$MgSO_4$	0.20 gm.
$Fe_2(SO_4)_3$	Trace
Egg-albumen (dissolved in sufficient N/10 NaOH)	0.15 gm.
Agar	15.00 gm.

As soon as the soil samples came to the laboratory, they were thoroughly mixed with a sterile spatula and 10 gm. transferred into 100 cc. of distilled

water. The mixture was shaken five minutes and further dilutions made with sterile tap water. The 10,000 and 100,000 dilutions were plated out in triplicates, on the modified albumen agar as a medium. The plates were incubated at 25°C. and, at the end of three days, the bacteria were counted; the plates were then incubated further to 14 days, when the actinomycetes could be counted. The modified albumen agar, being poor in nutrients, does not allow any rapid-growing organisms, such as molds and certain bacteria, to overgrow the plate. A certain portion of the soil was air-dried and the numbers were figured on the air-dry basis. The numbers present an average of all the plates used.

The data are given in table 1.

TABLE 1

Numbers of bacteria and actinomycetes in the soil developing on albumen agar

	SOIL TYPE	BACTERIA		ACTINOMYCETES	
		Numbers	Per cent	Numbers	Per cent
1	New Jersey Sassafras garden	5,300,000	85.5	900,000	14.5
2	New Jersey orchard	4,800,000	86.6	700,000	13.4
3	New Jersey clay meadow	8,100,000	93.7	550,000	6.3
4	New Jersey Sassafras forest	610,000	84.7	110,000	15.3
5	Iowa Carrington loam	1,764,000	88.2	236,000	11.8
6	Jamesburg Cranberry soil	204,500	96.5	7,500	3.5
7	Louisiana sandy loam	8,300,000	83.0	1,700,000	17.0
8	California fertilized soil	3,570,000	85.0	630,000	15.0
9	California unfertilized soil	580,000	63.7	330,000	36.3
10	California upland	2,220,000	64.2	1,238,000	35.8
11	California adobe	3,620,000	78.0	800,000	22.0
12	California sandy loam	6,010,000	80.8	1,430,000	19.2
13	Oregon adobe	13,100,000	84.6	2,400,000	15.4
14	Oregon white land	3,400,000	91.9	300,000	8.1
15	Porto Rico clay loam	2,140,000	69.0	960,000	31.0
16	North Dakota wheat soil	2,067,000	68.9	933,000	31.1
17	North Dakota flax soil	1,737,000	86.8	263,000	13.2
18	Hawaiian pineapple soil	4,334,000	86.7	666,000	13.3
19	Alaska soil	6,034,000	79.4	1,566,000	20.6
20	Texas Lufin fine sandy loam	2,126,000	78.7	574,000	21.3
21	Colorado alfalfa soil	2,440,000	61.0	1,560,000	39.0
22	Maine Aroostook potato soil	4,650,000	94.9	250,000	5.1
23	Maine dark Aroostook infected	15,900,000	87.8	2,200,000	12.2
24	Alberta grass soil	1,110,000	59.4	760,000	40.6
25	Alberta garden soil	2,000,000	54.0	1,700,000	46.0
	Average	4,245,000	83.0	870,500	17.0

The average of 25 soils taken in different parts of the country gives 4,245,000 bacteria per gram and 870,500 actinomycetes, the latter number representing an average of 17 per cent of the soil flora, exclusive of fungi. There does not seem to be any correlation between the climatic conditions and the number

relationships of the bacteria and actinomycetes. For example, the five most northern soils give different results, the Alaska soil giving 20.6 per cent of actinomycetes, or near the average; the two Maine soils giving 5.1 and 12.2 per cent, which numbers are below the average, while the Alberta soils gave 40.6 and 46 per cent, or much above the average. Among the southern soils we find the Hawaiian with 13.3 per cent, below the average; Porto Rican with 31, almost double the average, and Texas soil, near the average. The fact that the relative numbers of the microorganisms do not depend upon the climatic conditions, but upon the soil type, soil treatment, fertilization and crops grown, is made clear when the soils taken at one locality are compared. The three New Jersey Sassafras soils, under the different treatments, and with widely different numbers of microorganisms, show a similar percentage, somewhat below the average, between the actinomycetes and bacterial numbers, while the fourth soil, the clay meadow, contained only 6.3 per cent of actinomycetes, which is less than a half the proportion in the other three soils.

The two California soils which differ only in fertilization, with larger numbers of microorganisms for the fertilized soil, have shown more than twice as many actinomycetes in proportion to the bacterial numbers in favor of the unfertilized soil, the latter containing 36.3 and the first only 15 per cent of actinomycetes. Of the two Maine soils, a much higher percentage of actinomycetes was found in the infected, than in the normal soil. Great differences in the percentages of actinomycetes and bacteria are also found in the two Oregon and the two Dakota soils. It is interesting to note that the two Alberta soils, one of which was for the four years under grass and the other cultivated, gave 40.6 per cent of actinomycetes for the first soil and 46 per cent for the second one.

On the whole, we find that the heavy soils and those rich in undecomposed organic substances contain large percentages of actinomycetes. Such are the two Alberta soils, which were only a few years under treatment, the Colorado alfalfa soil, the North Dakota wheat soil and the Porto Rico clay loam. Of the three New Jersey Sassafras soils, the forest soil, although unfertilized and of a more acid reaction, which is unfavorable to the growth of actinomycetes, had the highest percentage of these organisms. This can doubtless be explained by the higher content of undecomposed organic substances. These observations bear out the ideas of Hiltner and Störmer (13), Fousek (8), and Conn (3). The differences between the California soils cannot be readily explained because of the insufficient information about those soils. The cranberry soil contained only 3.5 per cent of actinomycetes. This is easily understood, since this soil is under water a large part of the year, and the actinomycetes require good aeration for their development; secondly, this soil is rather acid, which, as will be pointed out elsewhere, is more or less inhibitive to the development of actinomycetes.

The isolation of types

Thirty species of Actinomyces were described by the writers in the first paper (25). Several of these organisms were thought to be the same as were isolated by Krainsky (14). As the comparison was made from descriptions only, no living cultures of Krainsky's organisms being at hand, the identification cannot be complete. Since the publication of the first article, the organisms have been kept in culture, and studied in different media. The further work brought out many more data, which will be presented at a later date. Over six hundred isolations of actinomycetes were made, many species having been isolated from several of the soils. Altogether about 110 distinct strains of actinomycetes were obtained, and this involved a large amount of work. The tendency was not to establish new species for those organisms that may vary slightly from others, but to form groups of organisms which possess common properties, although differing in certain details. In many cases groups were found to run into one another, so that it was often hard to tell whether a certain organism should be placed with one group or with another. The largest and the most troublesome group to work with is the *chromogenus* group, about 15 representatives of which were obtained. Great care has to be exercised in the study of the actinomycetes, and the *chromogenus* group in particular, because organisms that look at first very distinct may prove on further study to be identical; on the other hand, organisms that are found at first to be closely related, may prove on further study to be quite distinct. Such mistakes are made very often, and the authors did not escape them. *Actinomyces violaceus-Caesari*, *A. violaceus-niger* and No. 45, classed at first with the *chromogenus* group, have proved on detailed study to be identical, so that this organism will be spoken of further as *A. violaceus-Caesari*. Further study of the *A. Lipmanii* and the *A. aureus* that were thought in the beginning to be groups of organisms rather than single species, proved that the original proposition was true.

A word should be said concerning the growth of actinomycetes on artificial media. Certain species become well adapted to artificial media and give an earlier and better development after several transfers; others degenerate or die entirely, especially when they are grown on the same medium. When the organisms have been transferred to liquid cultures, soil, or a cellulose medium and then retransferred to Czapek's agar, many of them were thus rejuvenated.

Attention must be called to the fact that the actinomycetes studied by us until now and reported in these papers were isolated from the soil with the use of only egg-albumen agar. Had other media been used for isolation, many other organisms would have doubtless been obtained. This was proven by the fact that several organisms refused to develop on Czapek's agar, while they grew well on gelatin and ammonium sulfate-cellulose media. They probably preferred gelatin and ammonium sulfate to sodium nitrate

TABLE 2

Distribution of actinomycetes in the soils studied

SOIL TYPE	*A. violaceus-ruber*	*A. violaceus-Caesari*	*A. purpeochromogenus*	*A. viridochromogenus*	*A. chromogenus* group	*A. exfoliatus*	*A. diastaticus*	*A. albus*	*A. alboatrus*	*A. reticuli*	*A. citreus*	*A. griseus*	*A. alboflavus*	*A. verne*	*A. albosporeus*	*A. Californicus*	*A. Lipmanii*	*A. Rutgersensis*	*A. aureus*	*A. Fradii*	*A. roseus*	*A. laven-tulae*	*A. Bobili*	96	120	128	161	168
New Jersey garden soil				+	+			+			+						+	+	+									
New Jersey orchard soil					+			+			+		+				+	+	+				+					
New Jersey forest soil																			+		+			+				
New Jersey meadow soil					+			+					+					+	+									
Iowa loam	+				+						+						+		+									
Louisiana loam					+	+											+		+									
California fertilized				+													+				+							
California unfertilized				+	+												+					+					+	
California adobe	+		+		+	+		+	+	+		+				+	+			+			+			+		
California upland		+			+	+				+			+	+	+													
California loam					+		+									+												
Oregon adobe				+	+			+			+	+					+		+									
Oregon white land				+	+													+			+							
Porto Rico loam				+																			+					
North Dakota wheat soil					+												+	+	+						+			
North Dakota flax soil					+												+	+	+									
Hawaiian soil					+												+		+							+		
Alaska soil					+												+		+									
Texas soil				+	+												+	+	+									
Colorado soil					+			+									+	+	+				+					

as a source of nitrogen. A detailed study of the nutrition of these organisms will be given elsewhere (24). Czapek's medium, used almost exclusively in the previous work (25) for the description of the actinomycetes, is very well suited for this purpose. It is a rather poor medium for the development of actinomycetes; first, because most actinomycetes do not produce invertase so that the saccharose cannot be used rapidly; second, the sodium nitrate is not as good a source of nitrogen as certain organic nitrogen compounds. But, just on account of these deficiencies, this medium allows a very good differentiation of the organisms as to their growth, pigmentation and particularly the production of aerial mycelium.

The distribution of the different species and groups of actinomycetes in the different soils studied are given in table 2.

As seen from table 2, the *A. chromogenus* group, *A. Lipmanii*, *A. aureus*, *A. Rutgersensis*, and the others to a lesser extent, are found in soils collected from different parts of North America and Hawaiian Islands, so that these organisms can truly be looked upon as characteristic of the soil.

SUMMARY

1. The numbers of actinomycetes and their relation to bacterial numbers in 25 soils of North America and the Hawaiian Islands were studied.

2. Heavy soils and those rich in undecomposed organic substances are, as a rule, relatively richer in actinomycetes than corresponding lighter soils or soils poor in undecomposed organic matter.

3. The average number of actinomycetes to the total flora of bacteria and actinomycetes, taking an average of 25 soils, was 17 per cent.

4. A cranberry soil which was acid and covered with water part of the time contained only 3.5 per cent of actinomycetes.

5. Many Actinomyces species were isolated from different soils of North America and the Hawaiian Islands, showing their general distribution.

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THE ORGANIC PHOSPHORUS OF SOIL

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Received for publication July 29, 1918

Sometime ago there was published from this laboratory (12) a method for the determination of organic phosphorus in the weak alkali extract of soil. Briefly, this method, which is a combination of the Forbes (5) magnesia mixture and the Emmet-Grindly (4) neutral ammonium molybdate methods, involves first the clarification of an ammonia extract of the soil with a high speed centrifuge; second, precipitation of the resultant clear solution with magnesia mixture; third, the extraction of the well washed precipitate with nitric acid; fourth, the precipitation of this extract with neutral ammonium molybdate; and finally, the determination of the phosphorus in this precipitate. This gives the total inorganic phosphorus in the ammonia extract, which subtracted from the total phosphorus contained therein, gives the phosphorus in organic combination. This method and the results obtained by it have recently been called into question by Gortner and Shaw (7). Their conclusion bearing upon this is as follows:

> Probably the greater part of the phosphoric acid present in humus ash is inorganic in nanature, being derived from colloidal clay and from phosphoric acid absorbed by the colloids present in the ammonia solution. No method of analysis has as yet been proposed which will distinguish between such absorbed P_2O_5 and organic P_2O_5.

The purpose of the work herein reported was to test the method further to determine, if possible, whether it actually differentiates between the organic and inorganic phosphorus, in soil. An attempt was made also to discover something of the nature of the organic phosphorus. What success we have had in this two-fold purpose will appear in the following pages.

It is difficult to summarize briefly the data Gortner and Shaw present, which leads them to the conclusion given above. The humus, as they prepared it, contained large amounts of ash, the eight mineral soils which were used showing from 6.7 per cent to 32.6 per cent. The method of preparation of the humus was as follows (6): 15 gm. of air-dry soil with or without previous extraction with 1 per cent acid was shaken continuously for one week with 750 cc. of 4 per cent ammonia. At the end of that time, the solution was decanted from the soil and successively clarified by the addition—first, of $1\frac{1}{2}$ per cent of potassium sulfate and then of about 2 per cent of ammonium carbonate. After filtering, the solution was evaporated and the humus determined in the

usual way. It will be apparent that not only, as Gortner points out, will high results be obtained for humus because of the long-continued extraction, but also that excessively large amounts of inorganic phosphorus will be dissolved from the soils, large amounts of clay will go into suspension, and finally considerable amounts of inorganic phosphorus will be hydrolysed from any organic compounds present containing that element. Gortner and Shaw believe that because of the high ash content of the humus and because of the high content of phosphoric acid in the humus ash (2.83 to 28.25 per cent) most of it must be in inorganic combination. We will grant that much of the phosphorus in the humus as they prepared it might be in inorganic form, but the same does not necessarily hold true when our method of procedure is followed. The reason for this will be given later.

Further data obtained by them are taken to indicate the non-existence of phosphorus in organic combination. The data show no consistent relation between the amount of phosphorus extracted from air-dry soil and from acid extracted soil and that extracted by 1 per cent acid. This, we believe, proves nothing more than that varying amounts of inorganic phosphorus, both soluble and colloidal, are extracted by the different methods, which, added to a relatively constant amount of organic in the ammonia extracts, leads to the divergent results. Another fact given considerable weight by Gortner and Shaw is that 11.19 per cent of P_2O_5 was found in the humus ash from a subsoil containing only 16 per cent as much humus as its surface soil, whose humus ash contained 18.26 per cent of P_2O_5. We confess that we fail to follow their argument here, nor do we when they state that 11.19 per cent of P_2O_5 in the humus ash of the subsoil is largely inorganic in nature "due probably to the phenomenon of selective absorbtion." It no doubt is largely inorganic in nature but it would be simpler and it appears to us more correct to say that it was simply dissolved from the soil. An analogy is drawn between the way the soil acts and the action of Lloyd's reagent (11). This latter material is a hydrous aluminum silicate which absorbs alkaloids completely from acid solutions but gives them up when the reaction is alkaline. It is suggested that possibly soil acts in the same way in regard to phosphoric acid. They then state that if that were true "all of the absorbed phosphoric acid would appear in the humus ash as humus-phosphoric acid and would be counted as organic phosphoric acid in Potter and Benton's method." The analogy of the critics of our method is so forced that little need be said in regard to it. Why should the material after extraction from the soil with ammonia be a perfect absorbent for phosphoric acid in acid solution when the soil alone is not? That soil alone is not, is born out by the fact that acid extracts large amounts of phosphoric acid from soil. Russel and Prescott (13) have shown that dilute acids extract large proportions of phosphorus which has been added to soil as sodium phosphate. They also show, and this may be pertinent, that the soil retained its absorbtive power for phosphoric acid after extraction of the humus with 2 per cent sodium hydroxide. At this point it will be well to consider in

more detail the conditions under which the organic phosphorus was determined by our method.

In our work already published (12), one part of soil was shaken with 4 parts of 1.5 or 2 per cent ammonia for 1 to 2 hours. After settling for a few minutes the supernatent liquid was centrifugated in a machine whose bowl was $4\frac{1}{2}$ inches in diameter. The bowl was run at a speed of 10,000 revolutions per minute. Since the publication of that work a machine whose bowl has a diameter of 2 inches and a speed of 30,000 revolutions per minute has been used. Since centrifugal force varies directly as the square of the speed and directly as the diameter, the centrifugal force of the new machine is about four times that of the old. In the original description (15) of the old machine, results are given showing an average of about 1.4 per cent humus ash in humus from loams and silt loams and 2.2 per cent in clay loams. We have confirmed these results in a general way and in the analysis of a typical Carrington silt loam rather high in organic matter an ammonia extract from 100 gm. of the soil obtained by the old centrifuge gave 0.0414 gm. of ash while the same amount of extract from the new macline gave 0.0243 gm. of ash. This is the only ash analysis we have made with the new machine but enough has been said to make it apparent that analogies should not be drawn from Gortner and Shaw's determinations on humus as they prepared it and from the results from this laboratory. These writers also entirely overlooked an experiment reported in our first paper where inorganic phosphorus was added to the ammoniacal extract of a soil. The extract was then subjected to the regular procedure for the analysis of inorganic phosphorus and a complete recovery of the added phosphorus was obtained. This experiment effectively answers, we believe, the contention that the soluble inorganic phosphorus would be absorbed and the contention that colloidal phosphate-bearing clays make up the "organic" phosphorus is answered by the low ash content of our extracts.

Gortner and Shaw make one more criticism of our paper which must be answered. In taking exception to our statement that 1 per cent hydrochloric acid did not dissolve organic phosphorus from a certain soil they state that if phytin were present calcium and magnesium would be liberated by the acid, thus permitting the free phytin to be dissolved, but our conclusion which was specifically limited to one soil was drawn from an actual experiment. The soil used was very low in organic matter. Since that time we have found that soils high in organic matter may give up 1 or 2 per cent of their organic phosphorus to the 1 per cent hydrochloric acid extract. But that little, if any, of this is phytin phosphoric acid is shown by an experiment reported later in this paper. In brief, it may be stated here that phytin, in quite large amounts, was added to soil and the soil then extracted with 1 per cent acid. No increase due to the phytin was found in the soil. It should be pointed out here that practically all of the known organic phosphorus compounds, with the exception of the lecithins, are appreciably soluble in dilute acids, even nucleic acid, whether in the presence of proteins or not.

Even though the positive experimental proof of the existence of organic phosphorus in soil is questioned, it would seem reasonable to believe that there would be considerable present. Some of the reasons for this belief are as follows. Bacteria are known to contain approximately 1.5 per cent of phosphorus (16), a large part of which is believed to be combined in nucleic acid. While the amount of living bacteria in soil would account for but little of the phosphorus, yet disintegration of the cells would probably not be complete after many generations of the bacteria had died. There is, at present, no way of telling how long it would take for the cells to disintegrate in the soil, but

TABLE 1

Phosphorus and carbon content of some Iowa soils

CLASSIFICATION	COUNTY	POUNDS PER ACRE OF 2,000,000 POUNDS	
		Phosphorus	Carbon
Drift soils:			
Carrington loam	Clinton	860	26,960
Carrington fine sandy loam	Clinton	740	12,580
Carrington fine sandy loam	Clinton	780	10,141
Carrington silt loam	Clinton	1,030	45,190
Carrington loam	Webster	1,226	66,452
Carrington loam (steep phase)	Webster	1,020	28,432
Shelby silt loam	Scott	1,000	34,160
Shelby loam	Scott	800	25,760
Shelby fine sandy loam	Scott	480	8,520
Shelby loamy fine sand	Scott	400	7,720
Loess soils:			
Memphis silt loam	Van Buren	1,095	32,570
Grundy silt loam	Van Buren	953	44,220
Muscatine silt loam	Scott	1,167	46,573
Lindley silt loam	Scott	480	24,240
Wabash silt loam	Scott	1,230	40,300
Wabash silty clay loam	Scott	1,740	118,960
Sarpy fine sandy loam	Scott	1,000	21,460
Sarpy silt loam	Scott	2,020	49,100
Sarpy loamy sand	Scott	840	17,920

while nucleic acid as a whole is readily attacked by bacteria (16) the pyrmidine half of the nucleic acid molecule is extremely resistant to the action of acid (10) and enzymes (9). Therefore, the continued activity of bacteria in the soil might easily result in an appreciable accumulation of the pyramidine dinucleotide. On the other hand, of course, there is the opposing action due to the taking up of the organic and soluble inorganic phosphorus as it is formed, or as it is added to the soil, to give insoluble phosphates. But these insoluble phosphates can pass through the cycle of organic phosphorus quite easily, as has been demonstrated from the fact that molds and bacteria readily utilize the phosphorus of quite insoluble phosphates for their life processes.

It is a matter of rather common observation that soils rich in organic matter have a comparatively high phosphorus content. There may be, of course, many exceptions to this, but in general it seems to be true. In table 1 appear a few results not heretofore published which the soil survey department of th station has kindly supplied us. These results are typical of many others which have been secured and are merely selected at random to illustrate the point we are making. The results published in the report of the Soil Survey of Bremer County of Iowa (14) and the various reports of the Soil Survey of the Illinois counties should be consulted in this connection (1). From an examination of these results it is strikingly evident that a high carbon content is usually accompanied by a high phosphorus content. This is certainly indicative, although not absolute proof of the presence of organic phosphorus in soil.

EXPERIMENTAL

Gortner and Shaw, it will be recalled, maintained that because ammonia extracted such large and varying amounts of phosphoric acid from acid extracted and non-acid extracted soils, that all of the phosphoric acid so extracted was inorganic. The same conclusion was drawn from the amounts of phosphoric acid extracted from a subsoil when compared with that from its surface soil. In order to gather evidence in regard to those points, the following experiment was carried out.

A sample of surface soil was drawn from humus plot no. 106 and then some of the subsoil, 3 feet below the surface, was taken. Care was taken that the subsoil sample contained no surface soil whatsoever. The sample thus collected was bright yellow in color, and contained practically no organic matter.

No mechanical analysis was made of the soils, but except for the organic matter in the surface soil they were not materially different. The soils were ground to pass a 100-mesh sieve and then part of each sample was extracted with 1 per cent hydrochloric acid. The acid-extracted and non-acid-extracted soils were then subjected to the regular procedure for the determination of organic phosphorus. The results are given in table 2.

It is observed that more organic phosphorus was found in the acid-extracted soil no. 167 than in the non-acid-extracted. This might be due to rendering phytin soluble in ammonia by first dissolving out the readily-soluble bases with the acid. The fact that practically no organic phosphorus was found by our method in the subsoil effectually answers the contention that the "organic phosphorus" is due to clay in colloidal condition.

In order to determine whether phytic acid would be extracted from soil by dilute acid, some barium phytate prepared by the method of Anderson (1) was added to soil which was then extracted with 1 per cent hydrochloric acid. The experiment was conducted as follows:

Two hundred grams of soil were shaken for an hour with 350 cc. of 1.1 per cent hydrochloric acid and 50 cc. of water. Similarly, 200 gm. of soil were

shaken with 1.1 per cent hydrochloric acid and 50 cc. of a very dilute acid solution of the barium phytate. After filtration, 200 cc. of the filtrate was analysed for total phosphorus. The barium phytate contained 16.54 per cent phosphorus and 1.23 per cent of this was inorganic. The results of the experiment are given in table 3.

If all the increase in phosphorus in the acid extract of the phytin-treated soil was due to the inorganic phosphorus in the phytin, only 57 per cent of the total inorganic acid added was extracted. Therefore, it probably is a safe conclusion from this experiment that negligible amounts of phytin are ex-

TABLE 2

Phosphorus content of soils

SOIL	PHOSPHORUS IN THE SOIL					PER CENT ORGANIC PHOSPHORUS OF TOTAL PHOSPHORUS
	Total	In HCl extract	In NH_3 extract	Inorganic in NH_3 extract	Organic in NH_3 extract (by difference)	
	per cent	*per cent*				
Surface (no. 167)	0.0490	0.0150	0.0231	0.0041	0.0190	38.7
Surface acid-extracted			0.0281	0.0068	0.0213	43.5
Subsoil	0.0368	0.0131	0.0091	0.0089	0.0002	0.5
Subsoil acid-extracted			0.0085	0.0082	0.0003	0.8
Surface (orchard)	0.0300	0.009	0.0105	0.0027	0.0078	26.0
Subsoil	0.0268	0.009	0.0096	0.0097	None	

TABLE 3

Extraction of phytin from soil by dilute acid

MATERIAL EXTRACTED	PHOSPHORUS ADDED TO SOIL IN PHYTIN		PHOSPHORUS EXTRACTED
	Inorganic	Organic	
	per cent	*per cent*	*per cent*
Soil no. 167			0.00148
Soil no. 167 with Phytin	0.00037	0.00495	0.00169

tracted from soil by 1 per cent hydrochloric acid, even when added in an amount about three times the amount of total phosphorus ordinarily dissolved by 1 per cent acid.

Nature of the organic phosphorus of soil

There have been a few successful attempts to isolate organic phosphorus compounds from the soil but the amounts have been small and the results somewhat uncertain. When it is realized that soil may contain numerous representatives of practically all classes of naturally occurring organic compounds besides soluble and insoluble inorganic compounds, the difficulties en-

countered are not surprising. It was therefore thought that perhaps some information regarding the nature and the availability as plant nutrients of the organic phosphorus of the soil might be gained by a determination of the rate at which organic phosphorus compounds were hydrolyzed by acid. As Jones (10) has recently published results on the hydrolysis of nucleic acid with 5 per cent sulfuric acid at 100°C., it was decided to work under the same conditions.

The work of Jones was first repeated in part, using quite different concentration of the nucleic acid. While there is every reason to believe that the hydrolysis of nucleic acid is a first-order reaction and that, therefore, the percentage hydrolyzed in a given time will be constant, yet it was decided to repeat the work, as it was thought that Jones' conditions might not be duplicated exactly.

Our method of procedure was as follows. Commercial nucleic acid was purified in the usual manner by precipitating three times with glacial acetic acid. After washing in alcohol and ether it was dried in a vacuum, over sul-

TABLE 4

Hydrolysis of nucleic acid with 5 per cent sulfuric acid at 100°C.

DURATION OF HYDROLYSIS	CONCENTRATION: 0.06 GM. PER 50 CC.	JONES' RESULTS: CONCENTRATION 1 GM. PER 20 CC. PER CENT HYDROLYZED
hours		
½	40.9	29.6
1	49.6	45.6
2	56.8	53.9
3	59.0	55.8
5	62.5	58.5
Total phosphorus	7.98	7.94

furic acid and sodium hydroxide. 1.2 gm. of this material was dissolved in the minimum amount of ammonia and then the solution diluted to exactly 500 cc. Then 25-cc. portions, containing 0.06 gm. of nucleic acid, were placed in a series of beakers. In two portions, total phosphorus was determined, in two others inorganic phosphorus, and the remainder were hydrolyzed for different lengths of time as indicated in the table. No inorganic phosphoric acid was found in the unhydrolyzed samples. Sulfuric acid was prepared of such strength that 25-cc. diluted to 50-cc. with water would make the diluted acid exactly 5 per cent; 25-cc. portions of this solution were added to the beakers containing the 25-cc. portions of nucleic acid. These were covered with watch-glasses, and all the beakers placed in an Arnold sterilizer previously brought to the boiling temperature. It took about 10 minutes for the temperature to come back to the boiling point and the time of hydrolysis was taken from the moment the temperature was within 1°C. of the boiling point. It was found by preliminary experiment that the temperature of 50-cc. of water lagged only about two degrees behind that of the sterilizer.

After removal from the sterilizer the solutions were immediately cooled somewhat and neutralized with ammonia, and then precipitated with magnesia mixture and the phosphorus finally determined by the Lorenz method.

The results are given in table 4, and for comparison Jones' results are calculated to the same basis and placed in the table. Jones used more periods of hydrolysis than are given in this table.

It is seen from an inspection of the results that our results are higher than those of Jones. It may be that our conditions were sufficiently different to account for the variation. At any rate, it is apparent that we have to do there

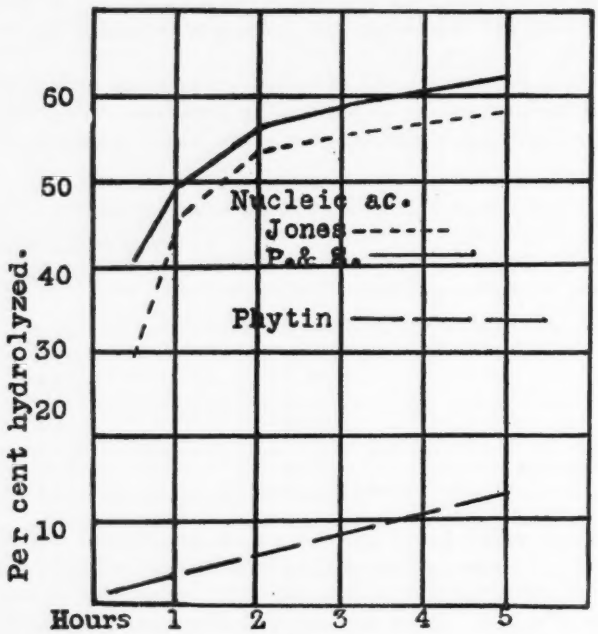

FIG. 1

with a first-order reaction. In figure 1 we have drawn the curves for both sets of results and it is seen that they are quite closely parallel.

An exactly similar experiment was carried out with phytin. The phytin used in this experiment was prepared according to the method of Clark (3) as outlined and modified by Boutwell (2). Because of the spontaneous hydrolysis of phytin the experiment was carried out immediately after preparation of the material. It therefore was not completely anhydrous. A solution of the phytin was prepared so that 25 cc. contained 0.03 gm. of phytin. Analysis of this showed 17.07 per cent of total phosphorus, which, corrected for a small amount of inorganic phosphoric acid found in it gives 16.9 per cent phytin

phosphorus. The results obtained by the hydrolysis experiment on this solution are given in table 5. Also, in this table the results obtained by the 5-hour hydrolysis of approximately twice the concentration of phytin are found. This experiment was made three days later than the other, and, therefore, while twice the weight of phytin was used, yet since it had been kept in a vacuum over phosphorus pentoxide the per cent of phosphorus was somewhat higher, being 18.055, of which 17.97 was found to be phytin phosphorus. In the calculation of the results correction was made for the small amount of inorganic phosphoric acid found in the hydrolyzed phytin.

The results from table 5 have been plotted and appear in figure 1. It is seen that the points fall fairly close to a straight line. Because of this fact and also because nearly the same percentage is hydrolyzed in the 5 hours when the concentration is double, this reaction belongs to the first order. The large divisions on the X axis in the curve for the hydrolysis of phytin make the points

TABLE 5

Hydrolysis of phytin with 5 per cent sulfuric acid at 100°C.

PHYTIN	DURATION OF HYDROLYSIS	PHOSPHORUS HYDROLYSED: PER CENT OF TOTAL PHYTIN PHOSPHORUS
grams	*hours*	
0.03	0 (blank)	0.788
0.03	½	2.040
0.03	1	3.050
0.03	2	5.460
0.03	3	8.220
0.03	4	10.070
0.03	5	13.050
0.06	0 (blank)	0.745
0.06	5	12.300

seem unusually close to a straight line, but it was desired to use the same percentage scale here as for nucleic acid.

In figure 2 it is observed that a curve appears for a "dinucleotide." Jones (10) states that all of the phosphoric acid is hydrolyzed from the purine half of the nucleic acid molecule in the first two hours, and therefore after two hours the curve is entirely due to the pyrimidine nucleotides. The results obtained by us on the hydrolysis of nucleic acid have been recalculated on this basis, the results of that calculation gives the curve mentioned above. It simply is the curve which one should obtain after the second hour if the pyrimidine dinucleotide has been started with.

In order to determine whether the organic phosphorus of the soil was hydrolyzed at rates at all analogous to the hydrolysis of these compounds of known constitution, experiments with several soils were planned. The following soils were used:

No. 1. Carrington silt loam. This soil was taken from no. 107 of the humus plots. It has been fallowed for ten years and has received no addition of any organic or other fertilizer materials.

No. 2. Miami silt loam I. This soil was taken from a station orchard. It is considerably lower in organic matter than soil no. 1.

No. 3. Miami silt loam II. Taken from a different place in the same orchard as the other Miami soil, and is somewhat lighter in color and lower in organic matter.

The method followed was to extract the soils with 1 per cent hydrochloric acid and then to determine the total and inorganic phosphoric acid content in

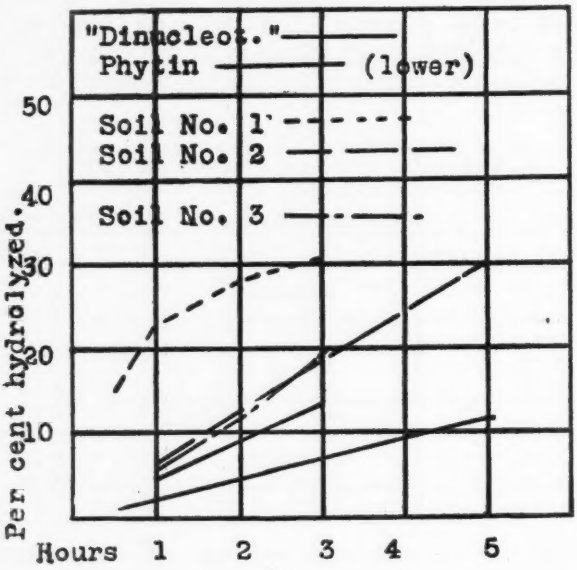

FIG. 2

the ammonia extract. One hundred cubic centimeters of a 1 to 4 extract was used in each case. Then other 100-cc. portions of the extracts were subjected to hydrolysis at 100°C., precisely as has been done in the case of the phytin and the nucleic acid. The results appear in table 6.

The results for the three soils have been plotted and appear in figure 2. As is observed the adjacent points for each soil have been joined by straight lines, as it was not thought that the accuracy of the data warranted the drawing of average curves.

Perhaps the most interesting thing to be noted on an inspection of the curves is the difference between the curve for soil no. 1 and soils no. 2 and 3. The curve for soil no. 1 more nearly resembles the nucleic acid curve, while the curves

for the soils no. 2 and 3 resemble the nucleotide or the phytin curves. Soil no. 1 is naturally higher in organic matter and more fertile, and hence bacterial activity is more vigorous, which perhaps accounts for this. All curves come close enough to the curves for phytin and "pyrimidine dinucleotides" to be perhaps significant, but, of course, from the meager data no definite conclusions can be drawn. While acid hydrolysis cannot be taken as an absolute criterion of availability yet it probably gives comparative results. If so, the organic phosphorus in the two orchard soils is rather unavailable compared with that of the Carrington soil. The results, we believe, indicate quite strongly that in none of these soils do we have much of the tetranucleotide, nucleic acid. The nucleic acid, if present at all, is probably mainly present as pyrimidine nucleo-

TABLE 6

Hydrolysis of organic phosphorus in soils with 5 per cent sulfuric acid at 100°C.

SOIL	TOTAL PHOSPHORUS (PER CENT OF SOIL)	DURATION OF HYDROLYSIS	ORGANIC PHOSPHORUS (PER CENT OF TOTAL PHOSPHORUS)	PER CENT HYDROLYZED OF ORIGINAL ORGANIC PHOSPHORUS
		hours		
No. 1	0.0490		43.5	
		½	37.0	15.00
		1	33.5	23.00
		2	31.4	27.80
		3	30.1	30.80
No. 2	0.0300		23.1	
		1	21.5	6.80
		2	20.1	13.00
		5	16.2	29.90
No. 3	0.0324		19.8	
		1	18.6	6.06
		2	17.5	11.50
		3	15.9	19.70

tides. This, of course, is not surprising, considering the quite highly-resistant character of this class of compounds. Phytin, also, while very readily attacked by enzymes which are usually associated with it, is relatively resistant to acid hydrolysis, and while it probably would be attacked by bacteria to some extent, yet the vigorous absorptive power of the soil for phytin as compared with the absorption of phosphoric acid as demonstrated by us, probably would indicate that the bacteria would by preference utilize the phosphorus of inorganic phosphoric acid.

SUMMARY

1. Gortner and Shaw's conclusion from their results does not necessarily hold for the results obtained by the use of our method for the differentiation of organic from inorganic phosphoric acid in the soil.

2. No "organic phosphorus" was found in subsoil containing no organic matter, but containing considerable amounts of colloidal clay. This proves that "organic phosphorus" is not due to colloidal clay, as is held by Gortner and Shaw.

3. Phytin, when added to soil, is not extracted to an appreciable extent with 1 per cent hydrochloric acid.

4. The hydrolysis curves for phytin and nucleic acid were found, the hydrolysis being effected with 5 per cent sulfuric acid at 100°C. The curve for phytin is a straight line and after 2 hours the curve for nucleic acid is approximately a straight line, thus proving both reactions to be of the first order.

5. The curves for the hydrolysis of the organic phosphorus of three soils were determined. Nothing definite could be drawn from the results, except that nucleic acid as such was evidently not present except in soil no. 1 and then to only a slight extent. The directions of the curves was such that the organic phosphorus might have been due to phytin or a pyrmidine nucleotide.

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See especially the Report of Tazewell County Soils, Illinois (8).